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The changing Phytophthora infestans population implications for late blight epidemics and control

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And Cell and Molecular Sciences

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Declaration

The results presented here are of investigation conducted by myself. Work other than my own is clearly stated with references to relevant researchers and their publications. I declare that the work presented here is my own and has not been submitted in any form for any degree at this or other university

Allison C. Chapman

We certify that Allison Chapman has fulfilled the relevant Ordinance and Regulation of the University Court and is qualified to submit this thesis for the degree of Doctor of Philosophy.

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Abstract

Phytophthora infestans, the causal agent of potato late blight, causes millions of pounds of losses worldwide each year. Within the UK *P. infestans* populations there has been a dramatic increase in the proportion of the A2 mating type due to genotype 13_A2 and concerns have been raised about whether this population change affects the reliability of the UK late blight forecasting system. The Smith Period is commonly used in the UK to predict blight risk on the basis of two criteria; on two consecutive days the minimum temperature must be 10°C or above and the relative humidity must be 90% or above for 11 hours on each of the days. The objective of this thesis is to examine the aggressiveness, competitiveness and response of contemporary UK genotypes to temperature and humidity. This was to understand more about what has driven the success of the 13_A2 lineage and how the population changes may have affected the validity of blight predictions based on the Smith Period. The laboratory studies did not provide conclusive evidence to explain the UK dominance of genotype 13_A2 as there were few consistent differences in aggressiveness or environmental response observed between genotypes. However, 13_A2 outcompeted other genotypes in the field trial showing that aggressiveness is best determined over multiple life cycles. The biological parameters tested showed that infection was seen at 6°C for 10 of the 11 contemporary UK genotypes tested. In whole plant tests, some infection was recorded after as little as 2 hours exposure to high humidity and after 6 hours exposure more than 10% foliar blight was seen. The Smith Period criteria thus need to be revaluated using up to date biological parameters of contemporary *P. infestans* populations to provide accurate prediction of potato late blight risk for growers.

Chapter 1 – Introduction

1.1 Potato late blight

Approximately 100 species of the genus *Phytophthora* have been identified and named, with *Phytophthora infestans* first being described by Anton de Bary (de Bary, 1876; Brasier, 2009; Kroon *et al*., 2012). The causal agent of potato late blight, *P. infestans* was the pathogen that contributed to the Irish potato famine in the 1840s (Bourke, 1993). Potato late blight is a devastating disease causing hundreds of millions of pounds worth of losses per year in Europe alone; this includes crop losses and money spent on late blight management (Haverkort *et al*., 2008). Even with the use of fungicides and other management methods *P. infestans* still remains a problem for growers.

1.2 Taxonomy of *Phytophthora*

P. infestans is an oomycete plant pathogen. It is defined taxonomically as belonging to the Kingdom Chromista, Phylum Oomycota, Order *Peronosporales*, Family *Peronosporaceae*, Genus *Phytophthora*, Species *infestans* (Birch and Whisson, 2001). *P. infestans* should not be mistaken for a fungus; it is grouped in a distinct Phylum Oomycota that are more closely related to the brown algae and diatoms than to fungi (Kamoun *et al.*, 1999; Smart and Fry, 2001; Judelson and Blanco, 2005). Oomycetes and fungi have common characteristics, such as filamentous growth, spore production and mycelia production but upon further inspection they fall into two different branches of the eukaryotic phylogenetic tree. Unlike fungi, oomycetes are diploid for the majority of the life cycle, produce lysine in different ways from fungi, and the major component of the cell wall is cellulose, unlike fungi, which use chitin (Latijinhouwers, 2003; Judelson and Blanco, 2005).

1.3 Host range

P. infestans infects a range of species from the genus *Solanum,* with the most economically important being the potato, *Solanum tuberosum,* but other species such as the tomato, *Solanum lycopersicum,* are also threatened economically. Non-crop host plants, for example nightshade species, could aid in the spread of *P. infestans* (Andersson and Johansson, 2003; Grenville-Briggs and West, 2005; Deahl *et al*., 2006).

1.4 The potato

The potato, *Solanum tuberosum*, is a part of the *Solanaceae* family. It is an annual plant that produces starchy tubers which are highly nutritious (Buckenhüsker, 2005). The potato originates from the Lake Titicaca region of the Andean mountains (on the border of Bolivia and Peru) where 8,000 years ago the native people domesticated the wild potato (Anon., 2008). It is thought that the potato was introduced to Europe in two ways. Firstly, during the Spanish invasion of Peru in the 16th Century, the Spanish conquistadors explored the Andean regions and upon finding the potato, promptly took it back to Europe. Secondly, it was brought from Venezuela to England by the slave trader John Hawkins (Anon., 2008; Buckenhüsker, 2005). The first recorded appearance of a potato outside of South America was in 1565 but it soon spread worldwide becoming an important food reserve (Anon., 2008). The potato is the third most important food crop in the world, behind rice and wheat and is grown in 130 countries worldwide with over a billion people using it as a food source (Schwartzmann, 2010). The potato is a hardy plant that can grow in a variety of climates and produce yield even if the soil and growing conditions are not optimal (Anon., 2008). In the UK during 2010 the total weight of potatoes produced was 3.9 million tonnes, with 2.03 million tonnes being treated with pesticides and some having multiple treatments (Garthwaite *et al*., 2010).

1.5 The origin and migrations of *P. infestans*

P. infestans was once thought to originate from the same region as its main host; the Andean region of South America. Records show that potatoes were cultivated and exported out of this region and evidence suggests that late blight had been present there for centuries (Andrivon, 1996; Abad and Abad, 1997; Gomez-Alpizar, 2007). For example, it was reported that in 1571, Jesuit Joseph Acosta observed that after cold weather in the Andes of South America potatoes were often found rotten in the ground (Neiderhauser, 1991; Abad and Abad, 1997). The Andean origin theory was widely accepted until the 1950s when the A2 mating type of *P. infestans* was found in the Toluca Valley in Mexico (Gallegly and Galindo, 1958; Neiderhauser, 1991). This spurred on a new theory of the origins of *P. infestans* as Mexico was the only location prior to the 1980s in which both the A1 and A2 mating types were found together. High levels of genetic diversity were found within Mexican populations due to the presence of both the mating types as sexual reproduction occurred (Tooley *et al*., 1985; Goodwin *et al*., 1992; Grünwald and Flier, 2005) and along with this, the resistance (R) genes that protected against late blight were found in *Solanum* species native to Mexico (Neiderhauser, 1991). Although many consider that Central Mexico is the centre of origin for *P. infestans* (Grünwald and Flier, 2005) molecular analysis of isolates originating in the Andes once again re-opened the debate (Gomez-Alpizar *et al*., 2007).

Wherever *P. infestans* originated, it is clear that a migration to Europe and North America occurred in the 1840s. This first migration apparently involved only a few genotypes and even though the genetic diversity of *P. infestans* was low, this did not hinder its spread (Fry and Goodwin, 1997). In Philadelphia, USA, reports of a new potato disease were published in 1843 and by 1845 *P. infestans* had spread across Canada, North East and Mid-West America (Fry, 1993). Many potato shipments were exported across the Atlantic to Europe and could have acted as a mechanism of

migration for *P. infestans*. First detected in Belgium in 1845, the devastating pathogen spread fast and was reported in Ireland around August/September 1845 (Bourke, 1993; Smart and Fry, 2001); Nelson (1995) stated that the first symptoms of blight in Ireland were found by David Moore, curator of the Botanic Gardens, Glasnevin, Dublin on the 20th August 1845. Potatoes were the staple crop of many in Ireland and once potato late blight arrived, it completely destroyed the food source on which the nation had become reliant. Between the years 1841 and 1861 the population of Ireland dropped by 2.4 million due to death and migration (Bourke, 1993; Duncan, 1999). A second pathogen migration, which probably began in the mid-1970s and was ongoing in the 1980s, came from Mexico and introduced new strains of both the A1 and A2 mating type (Sujkowski *et al.*, 1994; Fry and Goodwin, 1997). The first reported case of an A2 mating type isolate outside Mexico was in Switzerland in 1981 (Hohl and Iselin, 1984) with subsequent reports throughout Europe; in 1981 in the Netherlands (Frinking *et al*., 1987), in Sweden in 1985 (Kadir and Umaerus, 1987) and in Poland in 1988 (Sujkowski *et al*., 1994). The first incidence of the A2 mating type in England and Wales was in 1981 (Tantius *et al*., 1986), in 1983 in Scotland (Malcolmson, 1985) and in 1988 in Ireland (O'Sullivan and Dowley, 1991). Spielman (1991) proposed that the migrating genotypes were displacing the current population.

1.6 UK populations

For the fifteen years prior to 2005, a low proportion of the A2 mating type was detected in the UK *P. infestans* population, with around 90% of the population being the A1 mating type (Cooke, D.E.L., personal communication). Cooke *et al.* (2003) sampled 500 isolates from potato crops and gardens to assess the genotype diversity of the Scottish *P. infestans* populations between 1995 and 1997. Both A1 and A2 mating types were found, with a fifth of the samples being of the A2 mating type; the A2 mating type frequency did not change greatly throughout the three years. It was found

that only 5% of the isolates of the A2 mating type were resistant to metalaxyl and it was suggested that the limited number of A2 isolates in the Scottish population was due to their sensitivity to metalaxyl (Cooke *et al*., 2003). Similar results were seen in England and Wales between the years 1978 and 1998 with the A2 mating type occurring infrequently in most years (Day and Shattock, 1997; Day *et al*., 2004). In Northern Ireland, Cooke *et al.* (1995) reported that between 1981 and 1993 only six of the 250 samples collected were of the A2 mating type, with the first report being an isolate obtained from a tuber in 1987; the A2 mating type made up 3% of the Northern Irish population, although no samples of the A2 mating were detected between 1995 and 1996 (Carlisle *et al.*, 2001) or 1998 to 2002 (Cooke *et al*., 2006). In 2003 and 2004, the A2 mating was still infrequent in the population in Great Britain with 5.2% of the population in 2003 and 10% in 2004 being A2 (Shaw *et al*., 2007). It was not until 2005 that a marked change in the frequency in the A2 mating type was seen.

1.6.1 Genotype 13_A2

Between 2003 and 2008, Cooke *et al*. (2012a) collected 4,654 samples across 1,100 blight outbreaks in the UK through the Potato Council Fight Against Blight scout network. Genetic fingerprinting based on simple sequence repeat (SSR) markers was used to categorise the samples into different genotypes. SSR markers are DNA-based simple sequence repeat (SSR) motifs within the genome of the organism that can be used to distinguish small genetic differences amongst isolates using developed markers to give a genotypic fingerprint (Lees *et al*., 2006). A novel genotype of the A2 mating type was recorded in July 2005 in seven UK potato crops and quickly displaced the other genotypes; this was categorised as genotype 13_A2 and it was subsequently found to have been present in isolates collected from the Netherlands and Germany in 2004 (Cooke *et al*., 2012a). Variants within genotype 13_A2 were found as more isolates of the genotype were collected; variants were distinguished on the basis of changes in

alleles of two highly polymorphic SSR loci (Cooke *et al*., 2012a). Field and laboratory experiments have shown that genotype 13_A2 is highly aggressive, can out-compete other genotypes and overcome resistance in potato cultivars (Cooke *et al*., 2012a). Isolates belonging to genotype 13_A2 were virulence tested against 11 different R genes to see which R genes could be overcome. Out of the 11 R genes tested, only R8 and R9 were not overcome, suggesting that genotype 13_A2 had a broader virulence range than other genotypes (Cooke *et al.*, 2012a). In order to get accurate resistance ratings, in the UK the Science and Advice for Scottish Agriculture (SASA) test new potato cultivars in field trials to assess the level of foliar bight resistance of each cultivar and then the data are communicated via the Potato Council variety database (http://www.potato.org.uk/seed-export/varieties) on a 1-9 scale of increasing resistance (Lees *et al*., 2012). With the change in the UK *P. infestans* population there have been reports indicating that the resistance ratings of some cultivars do not match the published ratings (Lees *et al*., 2012). Lees *et al*. (2012) conducted a study on 49 existing potato cultivars and 42 new cultivars in six trials that spanned over 2008 and 2010 both at SASA and The James Hutton Institute. Most of the existing cultivars tested had resistance ratings that decreased when inoculated with genotype 13_A2; cultivars with historic resistance ratings of between 5 and 8 were reduced by 1 to 3 resistance points (Lees *et al*., 2012). The resistance ratings of some cultivars grown in the UK decreased; Cara was originally rated at a 7, but when inoculated with genotype 13_A2 the rating reduced to 5.5, Estima dropped from 5 to 3.3 and Lady Balfour dropped from 8 to 4. For Lady Balfour and Stirling, this decrease was due to the use of genotype 13_A2 as the resistance rating of these cultivars was higher in previous trials that used A1 mating type isolates (Lees *et al*., 2012). The decrease in resistance ratings could be due to different causes e.g. for cultivars Lady Balfour and Stirling the decrease was so large that it was most likely that genotype 13_A2 overcame the resistance that

remained effective against other isolates, whereas for cultivars that had small decreases in resistance ratings this could be attributed to the increased aggressiveness of genotype 13_A2 (Lees *et al*., 2012). Previous work conducted by White and Shaw (2010) clearly showed a reduction in the resistance ratings of several cultivars when inoculated with genotype 13_A2. Whole plant tests and a field trial reported by Cooke *et al* (2012a) in 2007 showed that the resistance of potato cultivars with partial resistance, such as Stirling and Lady Balfour, was lost when exposed to genotype 13_A2. Stirling contains at least one major gene for resistance, as well as genetically separable levels of quantitative field resistance. The proportion of genotype 13_A2 within the *P. infestans* population caused a dramatic increase in the A2 mating type which rapidly rose from 12% in 2005 to 72%, 78% and 67% in 2007-2009 (Cooke *et al*., 2012a).

1.7 Epidemiology

Potato late blight lesions can be found on the foliage, the stems and in the tubers of the plant. The first lesions within a crop are small spots that turn dark brown or black, lesions spread across the leaf and sporulation occurs on the outer edges of the lesion (Henfling, 1987). Sporulation occurs on the underside of the leaf and resembles white fluff or mildew-like growth (Henfling, 1987, Fry *et al*., 2001). Further into the epidemic, lesions are seen on upon leaves of the crop, particularly on leaf tips and crops may have a distinct ‘blight’ odour (Henfling, 1987). Lesions can be seen on stems, either from direct infection or from lesions spreading from the leaves; causing stems to weaken and collapse (Henfling, 1987). Tuber blight is characterised by firm brown lesions on the skin which penetrate into the tuber or remain superficial (Henfling, 1987; Fry *et al*., 2001). The relationship between foliar blight and tuber blight is not straightforward. *P. infestans* infects tubers via the buds, lenticels and wounds and the tissue inside the tuber is characterised by being dark brown (Porter and Johnson, 2004).

Tubers in storage with late blight are more prone to other diseases such as soft rot (Henfling, 1987).

1.7.1 Asexual life cycle

Depending on the temperature two types of germination of sporangia can occur. Direct germination is where the sporangium forms a germ tube to penetrate the leaf; this occurs at temperatures of between 20-25°C (Fry, 2008). However, at temperatures between 10-15°C sporangia germinate indirectly by releasing six or more zoospores which is the predominant method of natural infection (Judelson and Blanco, 2005; Fry, 2008). Zoospores are released from sporangia and provide multiple chances of infection. Water is needed for the zoospores to swim, which will only be over short distances, but passive movement is possible over long distances (Hardham *et al*., 1991). Zoospores have just a plasma membrane with no microfibrillar cell wall and use a water filled vacuole to osmoregulate by actively removing water so they do not burst (Hardham *et al*., 1991). The pathogen therefore favours moist conditions for the production of sporangia and zoospore release. Zoospores are uni-nucleated, biflagellate cells (Latijnhouwers *et al*., 2003) and physical or chemical signals promote flagella loss in the zoospores so that encystment can occur (Judelson and Blanco, 2005). The zoospore adheres to the host, forming a germ tube with an appressorium which may use a mixture of cell wall degrading enzymes and pressure to break through the cuticle of the leaf (Latijnhouwers *et al*., 2003); this can all occur within a few hours (Judelson and Blanco, 2005). Once penetration has taken place, the mycelia grow through the leaf tissue and in some instances haustoria start to form; haustoria are specialised hyphae that pierce through the cell wall but not the plasma cell membrane and expand inside the cell causing invagination of the host plasma membrane, and creating a space called the extra-haustorial matrix between the cytoplasm and the plasma membrane. This haustorial structure presumably allows *P. infestans* to use nutrients produced by the

plant for its own benefit (Latijnhouwers *et al*., 2003). *P. infestans* is a hemi-biotroph that has an initial biotrophic phase during which there are no symptoms and progresses into a necrotrophic phase when a lesion appears three to four days after infection. As *P. infestans* grows through the leaf tissue, the necrotic areas grow larger and sporulation occurs four to six days after infection (Fry, 1998). The sporangia form on the underside (abaxial) surface of the leaf as the sporangiophores grow through the stomatal openings. Sporulation normally occurs during the night (Judelson *et al*., 2009). Sporangia can be dispersed aerially for several miles or may be washed into the soil via water droplets and then go on to infect the tubers of the potato plant (Henfling, 1987; Fry, 1998).

The survival of mycelium and sporangia is affected by various environmental factors, such as temperature, radiation and humidity. Sunseri *et al*. (2002) suggested that there was a 4 hour time frame during which most infections occur; a total of 25 out of 566 sporangia infected after being exposed to direct sunlight and 23 of these were exposed for 4 hours or less. Similarly, for genotype US-8, Mizubuti *et al*. (2000) found that a 3 hour sunny period reduced the germination rate. The time frame of 3 or 4 hours would give sporangia ample time to disperse in windy conditions, but spread is limited by the effect on viability of UVB radiation (Jeger and Pautasso, 2008). Sunseri *et al*. (2002) also stated that the maximum survival time of sporangia was 24 hours when sporangia were in a shaded area and at 15°C. Minogue and Fry (1981) stated that between 15°C and 20°C the sporangia survive approximately 6 hours. Dried out sporangia are vacuolated and damage to the cell wall is often seen (Warren and Colhoun, 1975) showing that humidity has a large effect on sporangia; viability is decreased with drying and attempting to rehydrate the sporangia did not return viability .

1.7.2 Sexual reproduction

P. infestans is a heterothallic organism, meaning that it has two mating types, identified as A1 and A2 and is capable of reproducing sexually and asexually (Fry, 2008). Mating type is independent of sexuality; both mating types can produce male and female gametangia (Galindo and Gallegly, 1960). The sexual cycle starts with the development of the male and female gametangia; the antheridia and oogonium respectively. These structures are induced by a hormone that is specific to each mating type, which then fuse to form an oospore (Hemmes and Bartnicki-Garcia, 1975; Ko, 1978). When both mating types were present in plant tissue, Frinking *et al*. (1987) found that after five days the gametangia could be seen, fertilisation was established after nine days and the oospores were completely developed after 15 days. Oospores are thick-walled structures that are formed within plant tissue and can last from months to years in the soil waiting to infect the host by producing a hyphal tube or a germ sporangium (Goodwin, 1997; Judelson and Blanco, 2005). Turkensteen *et al* (2000) suggested that oospores formed in experimental potato and tomato plots on clay and sandy soils were still viable after 34 and 48 months. In order to infect, the oospores must germinate and form sporangia. Oospores present an additional problem in the control of blight as they provide a new source of inoculum (Drenth *et al*., 1995) that may contaminate soil and lead to earlier epidemics and of recombination, that could result in more aggressive strains (Brurberg *et al*., 2011). Nordic *P. infestans* populations show a diverse range of genotypes with no one genotype dominating (Brurberg *et al*., 2011). This suggests that more sexual reproduction is occurring as opposed to asexual reproduction (Brurberg *et al*., 2011). Genetic recombination, which occurs during sexual reproduction, recombines the alleles present in the gene pool; it will generate a more genotypically diverse population (Gavino *et al*., 2000). Self-

fertility in *P. infestans* in which a single individual produces an oospore has also been reported (Smart *et al*., 2000).

1.8 Climatic factors affecting epidemics

Temperature and humidity influence the progression of an epidemic by affecting the physiological and metabolic rates of *P. infestan*s. Germination, infection, colonisation, sporulation, dispersal and sexual reproduction are all affected by these climatic factors. For example, Becktell *et al*. (2005) examined the effect of temperature and leaf wetness on pathogen establishment (germination, infection and colonisation) for genotype US-17 on petunia and tomato and found that both factors had a strong influence. A two hour period of leaf wetness was needed for US-17 to begin establishing itself on the host with most occurring after a 6 hour period of leaf wetness. Time to infection and time to sporangial development was the shortest at 23°C and 28°C respectively, with the production of sporangia being greatest at 18°C. Although this experiment was not conducted on potato plants it still gives a good example of how climatic factors influence epidemics by affecting the incubation period (time taken from inoculation to infection) and latent period (time taken from inoculation to sporulation).

1.8.1 Temperature and the asexual life cycle

Temperature directly affects the growth of *P. infestans*, with the optimal temperature for the pathogen being approximately 20°C (Crosier, 1934). *In vitro* growth on oatmeal-dextrose agar showed an increased rate as temperatures were increased, but growth was hindered above 25°C (Crosier, 1934). The production of sporangia was optimal at 21°C but formation was still seen between 3°C and 26°C (Croiser, 1934). In Harrison's (1992) review it was stated that the optimal temperature range for sporangial production is 19-22°C, with the maximum temperature being 26°C and the minimum being 8.5°C. The direct sporangial germination rate increases with temperature

between 6°C and 24°C, but above 24°C it decreases, with the extremes of 3°C and 28°C inhibiting direct germination (Sato, 1994b). The highest rate of direct germination was between 22°C-24°C (Sato 1994b). Crosier (1934) found that no direct sporangial germination occurred at all at 30°C; whereas, Sato (1994a) stated that direct germination occurred at temperatures as high as 33°C. The optimal temperature for indirect germination was reported as 12°C to 13°C (Crosier, 1934), whereas Sato (1994a) saw indirect germination up to 20°C after which a decrease in germination rate was seen. Motility of zoospores is also affected by temperature, with zoospores swimming for 24 hours at 1°C but for only a period of 15 minutes at the higher temperature of 24°C (Crosier, 1934). More recently, Bain and Convery (2012) tested the optimal conditions for indirect germination for eight genotypes including genotype 13_A2 and genotype 6_A1 and stated that overall, the optimal temperature was 6°C but the differences between the genotypes were small. Shaw *et al.,* (2006) found similar results, showing the optimal temperature was between 4.5°C and 8.8°C. Genotype 13_A2 showed a clear optimal temperature at 8°C, whereas, genotype 6_A1 had a broader range at which it produced the most zoospores (Bain and Convery, 2012).

1.8.2 Temperature and oospores

The ability of oospores to survive in adverse conditions makes them a source of long-term inoculum (Fay and Fry, 1995). Fay and Fry (1995) examined optimal temperatures for germination by producing oospores and assessing germination at a wide range of temperatures. One thousand oospores were incubated at -80°C, -20°C, 0°C, 24°C, 30°C, 40°C, 43°C, 46°C, 49°C, 50°C and 60°C for either 2, 4 or 6 hours. After 9 to 14 days the number of germinated oospores present and the percentage germination was recorded. Germination was dependent on the parental lines. Most crosses showed no germination after being incubated at -80°C or 45°C and above for 2, 4 and 6 hours but at mid-range temperatures germination was seen in all crosses.

Conversely, Drenth (1995) found oospores could survive, and go on to infect, after being incubated for 48 hours at the temperatures 80°C to 35°C but viability was lost at 40°C. Oospores produced from crossing UK *P. infestans* isolates survived for 5 and 7 months in the soil when incubated at 0°C to 20°C and when stored in soil from a field (Pittis and Shattock, 1994). The temperature range at which oospores are produced is 8°C-23°C with the maximum production at 8°C (Cohen *et al*., 1997).

1.8.3 Temperature within the crop

Temperature has a major influence on both plant and pathogen growth and the progress of late blight epidemics. The temperature gradients within and above a potato crop change as the plants grow, for example, fields that had exposed soil had a higher within-crop temperature than fields that had a lot of foliage (Hirst and Stedman, 1960). Broadbent (1950) recorded the temperature within a potato crop for 3 seasons between 1947 and 1949. In 1947, measurements taken within the crop on dry and sunny days indicated, the 'within-crop' temperature was higher than the ambient temperature. Between 1948 and 1949, temperatures within the crop were measured at 10 cm, 20 cm, 30 cm and 60 cm above ground level. During cloudy days, there were only slight differences between the within-crop temperature and ambient temperature but on sunny days the differences in temperature depended on the canopy density. In an open crop, the largest differences between the within-crop and ambient temperature were seen at 10 cm, but in a dense crop the largest difference was seen at 30 cm. Soil wetness caused the lower levels of the canopy to have a lower temperature. Forecasting systems need to take into account the effect of canopy structure on the temperature to which the pathogen is exposed to within the crop.

1.8.4 Humidity

Relative humidity (RH) is the amount of water vapour in the air and it is expressed as a percentage of the amount needed for saturation (100%). The amount of water vapour that can be absorbed into the air varies with temperature. Higher temperatures allow more water vapour in the air so at 10°C and 100% RH there will be less water vapour present in the air than at 20°C and 100% RH. At very high RH levels dew will form on the surface of the crop. Leaf wetness is the amount of free water that is present on the surface of the leaf and this occurs after rainfall or when dew is formed. Leaf wetness directly affects the RH around the infection point and infection itself. In fact, Rotem *et al*., (1970) found that the optimal and minimum temperatures at which infection can occur depend on the leaf wetness duration.

1.8.5 Humidity and infection and sporulation

The presence of water is critical for the life cycle of *P. infestans*. The process of infection is most sensitive to the lack of water during the first three hours. If there is a two hour break in the leaf wetness during this period the number of sporangia that are able to infect the leaf reduces noticeably, but after the first 3 hours infection is affected to a lesser extent (Hartill *et al*., 1990). Once inside the leaflet, mycelial growth is not affected by humidity (Harrison and Lowe, 1989). Humidity and wind speed have a significant effect on sporangial production with the most sporangia being produced when the RH was very high, but during times of low wind speed the RH could be lower and sporangia production would still occur (Harrison and Lowe, 1989). Humidity is constantly changing within crops and this affects sporangial production, Harrison and Lowe (1989) found that when exposing inoculated detached leaflets of potato cultivar Bintje to alternating 13 hours of 80% RH and then 12 hours of 100% RH for eight days, the average number of sporangia formed was 31,370 compared to the 185 and 90,982 formed when incubated at a constant 80% and 100%, respectively. These results were

comparable to the whole plant tests which showed the number of sporangia produced at 100% RH was 75,793 compared with the 6,177, 0, 0 and 64 produced at 95%, 90%, 85% and 80% RH.

In the 1930s, Crosier (1934) conducted a series of studies looking at how humidity and temperature affect the viability of sporangia. Whole plants were incubated at either 15°C or 18°C at high humidity so that sporangia were produced; the plants were then placed into the closed chamber set to 20°C at seven different RH; 50%, 60%, 70%, 80%, 90%, 95% and 100%. Sporangia were collected every 30 minutes and allowed to germinate. Results indicate that sporangia cannot tolerate dry conditions; viability reduced as the exposure rate to lower RH levels increased. A lowering of RH is a critical step in sporangial release (Naerstad *et al*., 2009) but subsequent drying of sporangia affects their viability. Minogue and Fry (1981) found that sporangia were more sensitive when rapid rehydration occurred with the mean germination being 0.5% compared to those that were rehydrated slowly with a mean germination of 29.6%. When sporangia were dried for one hour at 20°C and 70% RH the mean germination was 32.5% for slowly rehydrated sporangia and 0.8% for more rapidly rehydrated sporangia. Death rate of sporangia was similar at 15°C and 20°C and there was no significant effect of RH between 40% and 88%. Once the sporangia are dispersed they can survive in the surface water for up to 21 days (Porter and Johnson, 2004).

1.8.6 Humidity and oospores

Studies have shown that a lower ambient RH is needed for optimal oospore production compared with sporangial production. Singh *et al*. (2004) inoculated potato plants with isolates of *P. infestans* and incubated them at 100% for 48 hours and a further four days for 80%. Once infection had established the plants were separated and put into three separate environments: the open air (19°C-22°C, 40-50% RH) or two growth chambers

set at 18°C and 50-55% or 82-90% RH). At intervals of 7, 14, 16 and 18 days oospores were counted from stem samples. Also, from field plots of cultivars Kufri Chandramuki, Kufri Jyoti and Kufri Badshah that were spray inoculated oospores were collected and counted at intervals of 5, 8, 13, 16, 24 and 30 days. Oospore production greatly depended on humidity and moisture content of the host tissue with a high moisture content of 88% being needed but with a low ambient RH. Plants in the open air environment formed oospores within 14 to 16 days, with the number of oospores increasing over time. When the RH was 80% no oospores were produced. Cultivar affected oospore production, with fewer oospores formed on the more resistant cultivars. The need for low RH for oospore production could coincide with the end of the potato growing season and the onset of autumn, thus triggering *P. infestans* to produce oospores to survive the winter.

1.8.7 Humidity in the crop

Humidity levels in the crop are affected by temperature and wind. Evaporation and transpiration from plants causes water vapour in the air. At the site of *P. infestans* infection on the leaf the RH is different from the ambient humidity around the crop as the water lost through transpiration increases the humidity around the leaf surface (Harrison, 1992). Two factors affect the humidity within the crop; the humidity surrounding the crop or the ambient humidity and the speed of air movement. Close to the leaf, transpired water evaporates into the air. Humidity at the leaf surface is dependent upon the rate of transpiration, stomatal movement and evaporation (Kitano and Eguchi, 1987). Water vapour travels slowly by diffusion but high air speeds remove the water vapour more efficiently (Harrison, 1992). The three major factors that affect infection on the leaf are humidity, wind speed and light as they influence the rate of drying of the leaf surface (Harrison, 1992). Zoospores need free water in order to survive and sporangia, although they can survive desiccation, must be rehydrated slowly

as rapid hydration is lethal (Minogue and Fry, 1981). In 1950, Broadbent studied the temperature and humidity changes within crops for three summers in 1947-1949. In 1947, the humidity and the dew point within the crop were higher in the day than at night. In the summers 1948 and 1949, records of the temperature, humidity and wind speed were taken more regularly at a number of different points in the crop. Humidity within the crop depended on various factors, for example, the humidity was highest in the lower layers of the canopy (10 cm off the ground) but if the soil was dry the humidity was highest at 30 cm off the ground. Humidity fluctuations in the crop were due to the wind and the amplitude of the fluctuation depended on the wind speed within and above the crop. In recent years, Jacobs *et al*. (2005) simulated leaf wetness in the canopy of a potato crop. The model was separated into three layers denoting different layers of the canopy; top, middle and bottom. Dew distribution was not uniform throughout the canopy. It changed over time depending on the layout of the leaves and the weather, for example, the upper layers of the canopy collected more dew than the lower layers but they were the first to dry out whereas the lower layers maintained the moisture for a longer period of time. Hirst and Stedman (1960) also noticed that the architecture of the canopy affected humidity within the crop. When they looked at fields that had exposed soil the temperature within the crop was warmer and the humidity was lower compared with fields that had more dense crops, while the denser crops retained a high RH for longer.

Irrigation affects the humidity within a crop. Olanya *et al*. (2007) assessed the RH in a potato canopy (cv. Russet Burbank) under four different irrigation regimes; sub-surface drip, sprinkler, surface drip and non-irrigated, for three years. The microclimate, which consisted of air temperature, soil temperature, leaf wetness, rainfall and RH, was recorded at hourly intervals by data loggers which were placed in the upper portion of the canopy. The leaflets in the canopy were the wettest during the night and the early

morning with leaf wetness and RH being the highest. The RH in the canopy was up to 10% higher after sprinklers and surface drip irrigation compared to the sub-surface drip irrigation and the non-irrigated plots in the first eight hours. After 12 hours the RH was over 90% in all irrigated plots. Irrigation had no effect on the temperature within canopy and soil temperature

1.9 Aggressiveness

Aggressiveness is the amount of disease that is caused by a pathogenic organism (Andrivon, 1993). It is affected by factors such as host resistance, temperature (Cooke *et al*., 2010) and fungicide resistance (Day and Shattock, 1997). Fitness measures the ability of an organism to survive, reproduce in the environment and contribute genes to the next generation (Orr, 2009). Aggressiveness and fitness are related but the relationship is complex. A pathogen does not necessarily have to be highly aggressive to be fit, for example, rapid colonisation of tubers, as reported by Kirk *et al.* (2001) for the US genotype US-8, would be beneficial for a genotype in the short term by allowing it to spread quickly. However, it may not aid long term survival of a specific genotype (i.e. its fitness), because in winter storage the tubers would be its only source of nutrients and if they rotted away completely it would be lost (Johnson and Cummings, 2009). However, in fact when Johnson and Cummings (2009) stored tubers infected with genotype US-8 at 4°C, the tubers did not completely rot, showing that this aggressive genotype could survive the winter storage period. Similarly, a study by Montarry *et al.* (2007) using French *P. infestans* isolates found that the level of aggressiveness did not make much difference to their over-winter survival. Both these studies suggest that a high level of aggressiveness is not a hindrance to survival of *P. infestans* genotypes i.e. genotypes can be both highly aggressive and fit. It has also been suggested that new *P. infestans* genotypes may be better able to infect alternative hosts and that expansion of the effective host range may be involved in pathogenic

changes in *P. infestans* populations (Deahl *et al.* 2006). Grönberg *et al.* (2012) suggested that alternative hosts may increase the number of aggressive isolates within the population. Isolates infecting hairy nightshade (*Solanum physalifolium*) had a shorter latent period and produced more sporangia than isolates collected from potato at the same site, so the more aggressive isolates from the nightshade will, in theory, out-compete the potato-restricted isolates. In contrast, isolates derived from the Cameroonian garden huckleberry (*Solanum scabrum*) were less aggressive than ones isolated from potato (Fontem and Olanya, 2008).

1.9.1 Variation in aggressiveness

Selection will act on natural genetic variation in the *P. infestans* population to increase the frequency of more aggressive genotypes or those with an ability to adapt to overcome partial host plant resistance. In 2009, the genome of a *P. infestans* isolate T30-4 was sequenced and was found to be 240 Mb in size (Haas *et al*., 2009). The size of the genome was compared with those of other *Phytophthora* species, for example *P. sojae* has a genome size of 95 Mb and *P. ramorum* has only 65 Mb. The *P. infestans* genome contains a large proportion of repetitive DNA and transposons, which are DNA sequences that can move around the genome. This could have aided the evolution of the organism and its ability to adapt rapidly to its host plants. The genome sequence also revealed large complex families of apoplastic and cytoplasmic effectors (Haas *et al*., 2009). One of the main families of effectors have a distinctive RXLR amino acid domain that allows them to enter host cells (Whisson *et al*., 2007) and are termed the RXLRs. The high rate of mutation due to the transposons and a large array of effectors found in the repetitive DNA of the genome could help to explain variation in aggressiveness within *P. infestans* populations.

Cooke *et al*. (2012a) sequenced the genome of the genotype 13_A2 isolate 2006_3928A and found that 95.6% of the genes in the genome were situated in the same place as those in the T30-4 genome that was sequenced previously (Haas *et al.* 2009). Cooke *et al*. (2012a) showed that of these, 99.2% of the coding regions in isolate 2006_3928A were identical to isolate T30-4 (Haas *et al*., 2009). Small genetic variations, termed single nucleotide polymorphism (SNPs), were examined and, in the isolate 2006_3928A, 22,433 SNPs were found in 5,879 coding sequences compared to T30-4. Of these 22,433 SNPs, 11,795 were unique to isolate 2006_3928A and represented nonsynonymous substitutions. A nonsynonymous substitution is one that leads to different amino acid being used in the protein that it codes for; synonymous substitutions do not affect the amino acid produced. Four hundred and five SNPs were detected in the RXLR genes of isolate 2008_3928A with 278 of these being nonsynonymous, meaning that isolate 2006_3928A had RXLR genes that were unique. Gene copy number variation was identified in isolate 2006_3928A and higher copy numbers of one RXLR gene were demonstrated for multiple isolates of 13_A2 compared to 18 other *P. infestans* isolates of different genotypes. Six novel RXLR genes were found in isolate 2006_3928A that were not present in T30-4 and during the biotrophic stage of infection 104 RXLR effectors were expressed in isolate 2006_3928A compared to the 79 found for T30-4 and 68 for the Netherlands isolate NL07434. Up-regulated genes were induced for a longer period of time in 2006_3928A which corresponded to a longer biotrophic phase when compared to the other two isolates in the test. It was hypothesised that this larger biotrophic lesion, that was growing faster and evading host detection, may be related to this genotype's aggressiveness and fitness in the field (Cooke *et al.*, 2012a).

P. infestans went through a genetic bottleneck when it was first introduced into Europe in the 19th century, meaning that the gene pool was dramatically reduced due to loss of

individuals (Fry, 1997). With the arrival and spread of the 'new' population in the 1970s to 1980s both A1 and A2 mating types were present in Europe. However, sexual recombination seems to have been a relatively rare event with single clones being dominant in many regions. Mutation is an important source of variation. Over the decades mutations would have accumulated and due to migration within Europe the pathogen may have adapted to the different climates and cultivars used in each region, thus giving rise to distinct sets of populations throughout Europe. Small mutations within genotypes produce variants, for example, genotype 13_A2_2 is the dominant 13_A2 variant in Scottish populations. Host-pathogen interactions also are a driving force in evolution, leading to more complex races of *P. infestans*. A high level of fungicide application also applies a strong selection pressure for resistance to fungicides to emerge. Within-genotype variation, due to mutation, would have led to different virulence factors being present in the genome and differences in parameters such as growth rate and latent period. Goodwin (1997) stated that the rate of mutation would not have to be excessively high to account for the observed levels of variation; the vast numbers of sporangia produced by a single infected field and the applied selection pressure of host resistance and fungicide application could create an environment that would positively select for the observed within-genotype variants. Mitotic recombination has been shown to occur in some *Phytophthora* species, such as *P. sojae* and *P. capsici* (Chamnanpunt *et al.*, 2001; Whisson *et al*., 2004; Lamour *et al*., 2012). Changes in the zygosity of loci reveal hidden recessive alleles that could give an isolate an advantage over others in the population allowing it to become dominant (Goodwin, 1997).

1.10 Competition

Competition between genotypes may be a factor that has contributed to the dominance of genotype 13_A2 in the UK population. Competition has been described as the

ability of an individual to inhibit another individual, whilst resisting inhibition itself (Newton *et al*., 1998) whereas fitness is the contribution an individual makes to the gene pool and the survival rate of that contribution (Tooley and Fry, 1985; Pringle & Taylor, 2002). The competitive ability of an individual is important in terms of fitness but it is not the only factor that makes an individual more fit than the others (Newton *et al*., 1998). Other factors such as aggressiveness, length of life cycle and environment play a part in determining fitness. It is important to remember that competition and fitness are not the same. Odling-Smee *et al*. (2003) stated that environmental conditions will have a differential effect on fitness for two individuals. For example, if isolate A could infect at a lower temperature than isolate B, isolate A's fitness would be different from isolate B's even though they were in the same environment. This would give each of the isolates a different niche within the population. A fundamental niche is defined as a set of environmental parameters that affects fitness but the biotic interactions are not taken into account (Hutchinson, 1957). Organisms tend not to inhabit the entire fundamental niche, but smaller parts called the realised niche which takes into account the biotic interactions (Pulliman, 2000).

Studies have been conducted on other plant pathogens to look at the effect of competition and fitness. Yang and TeBeest (1995) tested, in growth chambers, the competitive abilities of wild type and mutant isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of northern jointvetch. They found that the mutant isolates were both less fit and less competitive. Disease components such as infection efficiency, lesion size and latent period were significantly smaller in the mutant isolates compared to wildtype. It was proposed that the decrease in the mutant population was due to the strong competition of the wildtype, but the mutant isolates did not die out of the population (Yang and TeBeest, 1995). Ditmore *et al*. (2008) conducted field and glasshouse experiments to investigate competition between two isolates of *C.*

gloeosporioides using either single or mixed isolate inoculations followed by a challenge inoculation (also with either single or mixed isolates) 4 days later. One isolate was dominant, comprising 80% of the population even after a challenge inoculation, and significantly reduced the number of lesions produced by the other. This suggests that the dominant isolate induced defence responses to stop others infecting.

Genetic characteristics could give a particular genotype a competitive advantage over others. For example, genotypes that produce a larger proportion of sporangia, release zoospores earlier, germinate faster, produce more haustoria and assimilate nutrients better would have an advantage. Pathogenic adaptation could be a reason behind competition between genotypes. A genotype that could infect other host plants could have an advantage over an isolate that is highly aggressive to potato but not on other host plants. For example, other *Solanaceae* host plants could act as a 'go between' from one potato field to the next. The isolate that can infect all host plants and is not adapted to any one host would be at an advantage here. Lebreton *et al*. (1999) measured the competitiveness of one potato and one tomato isolate. At two locations, potatoes of cv Bintje were artificially inoculated with the two isolates by introducing plants that were already infected and had sporulating lesions into the plots. Disease severity was assessed at two different dates at each location and from each plant an infected leaf was collected, isolated and characterised. The potato isolate showed greater competitive ability than the tomato isolate on the potato plants, but some isolates were less fit on potato than on tomato when fitness was based on lesion size alone. Pathogenic adaptation to a host could explain the difference in the population between potato and tomato fields, but it is vital to take into account factors other than lesion size when estimating fitness; sporulation, infection efficiency and climatic factors are also important. Tooley and Fry (1985) investigated spread of *P. infestans*

isolates at two locations by inoculating the central plant in potato field plots and assessing infection. There were differences in disease spread at the two locations associated with environmental differences, and differences in fitness among isolates were found at one location, but not the other. Where differences in fitness were found these were correlated with levels of disease suggesting that the isolates that caused the most disease were the fittest. However, using different methods of calculating fitness different results were given, for example disease assessment data gave larger fitness estimates than population size data.

The recent population changes have given rise to a new population of genotypes that are fitter than the old population. The 'old' population refers to the isolates that were in the population before the discovery of the A2 mating type in Europe, the 'new' population refers to the new isolates that were discovered after the introduction of the A2 population, but contains both A2 and A1 mating types (Spielman *et al.*, 1991). Young *et al.* (2009) looked at how the different levels of resistance between cultivars affect selection of *P. infestans* populations. Six genotypic groups of isolates were used to infect field trials consisting of four cultivars. Visual assessments of disease were conducted every 4 days after first infection had occurred and samples of individual plants were taken. It was found that the most resistant cultivars had fewer lesions than the others. In Northern Ireland, the different groups of isolates used dominated different cultivars, whereas in Michigan, it was found that US-8 dominated the population. Miller and Johnson (2000) investigated the competitive ability of the old (US-1) and the new populations (US-8) by introducing infected plants into experimental plots. They found that US-8 was fitter than US-1; it was the dominant genotype in the population, with only a few US-1 isolates recovered. The lesion expansion of US-8 was significantly larger than that of US-1; this could affect the infection of US-1 as US-8 would colonise more parts of the plant, leaving fewer healthy tissues for US-1 to infect.

Miller and Johnson (2000) also suggested that if the sporangia of US-8 germinated faster than US-1, this would allow US-8 to infect more frequently than US-1. Isolates from both genotypes were capable of causing disease but once US-8 was established the other genotype had trouble infecting. Competition between genotypes could also be related to the infection process. It is possible that one genotype could overcome more R genes making it a better competitor on cultivars containing R-genes or once infection had taken place it might inhibit subsequent infections, although since most commercial cultivars are susceptible this is not significant in practice.

1.11 Blight management

P. infestans causes major crop losses for growers; once a field is infected there is no stopping the spread of the disease. The number, or size, of tubers produced may be significantly reduced, if the tubers become infected they are unsuitable for marketing and blighted tubers that are introduced into store undetected usually rot. Elimination of the primary inoculum is the first step to control blight and vigilance is the key. Regular scouting of potato fields allows any infected potato plants to be spotted and destroyed. Piles of unwanted or waste tubers, commonly known as cull piles, can harbour infected tubers and aid the spread of late blight into the next growing season, particularly if situated next to a field (Henfling, 1987). Infected waste should not be stored with the new crop and should be covered to prevent spread (Cooke *et al*., 2011). It is important not to save potato seed from blighted crops for the next growing season and to plant only non-infected seed tubers (Fry, 1998). Using certified seeds helps to eliminate primary inoculum, although this is not a guarantee (Fry *et al*., 2001). The sale of seed potatoes is controlled by regulations and in order to be certified it has to satisfy inspection standards (Cooke *et al*., 2011). All seed and tubers should be examined carefully and rejected stock should be discarded (Fry *et al*., 2001). Simple crop care measures may also be useful such as, planting field plots further apart aids to reduce

spread and avoiding use of sprinkler irrigation which promotes infection (Henfling, 1987).

1.11.1 Host resistance to *P. infestans*

Plants have adapted many ways to defend themselves against pathogens, from waxy cuticles to intra-cellular proteins, but plants and pathogens are still in an evolutionary 'arms race' (Holub, 2001). Pathogens enter host plants via natural openings, wound areas and by penetrating host tissue (Jones and Dangl, 2006). Once inside, exploitation of the host begins, but plants have several defence mechanisms that protect against pathogen attack. Race specific resistance is the single gene resistance specific to a pathogen; it is also known as vertical resistance (Peters *et al*., 1999). Processes like the hypersensitive response (HR), which is a genetically regulated form of cell death that occurs in metabolically active cells (Mur *et al*., 2008), expression of pathogenesis-related (PR) proteins, which are anti-microbial proteins, and the oxidative burst, which involves the generation of reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-), which are reduced molecular oxygen, are all involved in race-specific resistance (Mysore *et al.*, 2004). The HR is triggered by avirulence factors, produced by the pathogen, that are recognised by resistance genes (R) in the plant. This is called the gene-for-gene concept (Flor, 1971). For example, Pentland Dell contains the R genes 1, 2 and 3, so in order for an isolate of *P. infestans* to infect, the avirulence factors which are recognised by the R genes present in Pentland Dell would need to be absent or mutated so as not to be recognised. Breeding for resistance using R genes from wild *Solanum* species began nearly 100 years ago (Gebhardt and Valkonen, 2001), but as *P. infestans* is highly adaptable its success depends on the genotypic variation in the population. For example genotype 13_A2 has led to a reduction in resistance ratings of potato cultivars (Lees *et al*., 2012). A variety of R genes can be present in the plant genome, consequently allowing greater protection

against pathogen attack. There are 11 known R genes from *S. demissum* which confer resistance to *P. infestans* in potato, some of which show slight variations, such as R_3 and R_4 giving rise to alternatives of those genes. In 1953, the nomenclature regarding R genes was standardised and in doing so four R genes were named: R_1, R_2, R_3 and R_4 (Black *et al*., 1953). R_5 and R_6 were identified in the 1960s by Eide *et al*. (1959), R_7, R_8 and R_9 were identified by Malcolmson and Black (1966) and R_{10} and R_{11} were identified by Malcolmson (1969). The recognition of avirulence factors causes a cascade of biochemical changes within the cell, such as rapid ion fluxes, production of reactive oxygen species (ROS), membrane disruption, cell wall cross-linking, and the accumulation of salicylic acid (Kombrink and Schmelzer, 2001) which stop the pathogen further infecting the plant. Non-race specific resistance is polygenic and provides a broad range of defence against many pathogens; this is also known as horizontal resistance.

1.11.2 Fungicides

Fungicides are an effective preventative measure to control late blight. Most of the potato cultivars grown in the UK are susceptible to late blight so the use of fungicides is essential, and potato crops receive up to 15 or more applications depending on the weather (Cooke *et al.,* 2011). Fungicides are costly, Erwin and Ribeiro (1996) stated that as much as 25% of the cost of fungicides worldwide is spent on control of *P. infestans*, and can have a detrimental effect on the environment so the need to optimise their usage is essential. Steps such as using the correct type of fungicide for the stage of the epidemic, timings of the sprays and using resistant cultivars may help to reduce the amount of fungicides used (Gans, 2003; Forbes, 2004). In the UK, growers apply fungicides regularly with a maximum interval of 7 days between each application regardless of the risk (Hansen *et al*., 2012).

1.11.2.1 Metalaxyl resistance

The drawback to reliance on such fungicides is the risk of selection of fungicide-resistant strains of *P. infestans*, as occurred with the phenylamides (Cooke and Little, 2010). Metalaxyl is an acylalanine phenylamide that is very effective against oomycetes. It was first used commercially in 1978 to control *P. infestans* (Cooke *et al*., 1991), but resistance to metalaxyl appeared in 1980 and led to control failures (Dowley and O'Sullivan, 1981). Shattock (1988) studied the inheritance of metalaxyl resistance by examining the mating progenies of *P. infestan*s isolates that were resistanct or sensitive to metalaxyl and stated that resistance was due to a single, incompletely dominant nuclear locus. Lee *et al.* (1999), who also studied the inheritance of metalaxyl resistance concluded that it was controlled by a single dominant gene modified by other genes of minor effect. Judelson and Roberts (1999), who studied North American, Mexican and Dutch isolates, proposed that there were at least two major genetic loci associated with metalaxyl resistance and concluded that a diversity of mechanisms contributes to phenylamide resistance. Metalaxyl resistance became common within *P. infestans* populations in the UK and Ireland. Holmes and Channon (1984) assessed *P. infestans* isolates on the south-west of Scotland and in 1981, out of the 63 crop sampled, 44% of the isolates collected were metalaxyl-resistant. Day and Shattock (1997) reported an increase in resistance between the years 1978 and 1995 in England and Wales. Dowley and O'Sullivan (1985) showed that when metalaxyl was not used in a region the levels of metalaxyl-resistant isolates reduced within the population; between 1981 and 1983, the levels of metalaxyl resistance reduced from 75% to 6% in Ireland.

1.11.2.2 Other fungicides

According to the review by Cooke *et al.* (2011) in Great Britain, fungicides accounted for 65% of the total pesticide-treated area of ware potatoes in the 2008 survey (Garthwaite *et al.*, 2008). Three formulations, cymoxanil+mancozeb, cyazofamid and fluazinam, accounted for over half of all fungicides applied. Fungicides were applied to over 1.6 million hectares, and these three formulations accounted for 23%, 20% and 16% of this area, respectively. Other fungicides which were widely used were propamocarb hydrochloride+fluopicolide, mancozeb, dimethomorph+mancozeb and mandipropamid. The effectiveness on different parts of the potato plant and the modes of action of the various fungicides available for late blight control have been summarised in the Fungicide Comparison Table published on the EuroBlight website (http://www.euroblight.net/FungicideComparison.asp).

A strategy to reduce the amount of fungicide used to control blight is needed as new EU regulations have been set out to reduce the risk to human and environmental health by reducing fungicide use (Anon, 2009). This has included prohibiting aerial spraying, reducing or prohibiting spraying in public areas and tighter regulations on fungicides that are harmful to aquatic habitats (Anon, 2009). Naerstad *et al.* (2007) conducted field experiments to look at the effect of different levels of host resistance in combination with fluazinam applications on control of late blight. Six cultivars with different levels of resistance were treated with 10 different fungicide treatments. The application intervals were 7, 14 and 21 days and the doses were 100%, 50% and 33% of the recommended dose of the fungicide Shirlan (150 g a.i./ha fluazinam). A foliar blight model was developed for this study to examine interactions between cultivar resistance and fungicide applications and this showed that using half the recommended dose of fungicide reduced the protection effect to 84% whereas extending the intervals between sprays reduced the protection effect to 59%. This model was developed to

understand the study's results and was not validated. Weekly fungicide applications on cultivars with low levels of resistance did not prevent foliar blight, but cultivars with higher levels of resistance showed low foliar blight levels on unprotected plots. This shows that specific fungicide regimes need to be made for cultivars with different resistance ratings.

1.11.3 Blight forecasts

The use of resistant cultivars and regular scouting for possible blight outbreaks helps to combat potato late blight outbreaks but disease forecasting is an important part of potato late blight management. Blight forecasts dependent on meteorological conditions are used to assess the risk of a blight outbreak (Taylor *et al*., 2003) and inform farmers when to use fungicides, but also to avoid wasting costly fungicides by using them only when needed (Hardwick, 2006). Application should be more frequent when conditions are more favourable for blight.

1.11.3.1 The Smith Period

Climate has an impact of the prevalence of potato late blight and patterns between epidemics and meteorological data can be used to create an empirical forecasting system. Beaumont (1947) studied four rules which stated when potato late blight would be a potential risk, allowing growers to be more aware of the date that fungicide sprays should be started. The rules were: that dew must occur for at least four hours during the night; minimum night temperatures must be 10°C or above; cloudiness on the day following the dew, and rainfall on the day following the dew, must be at least 0.1 mm. Although this system worked well in Holland, difficulties arose when trying to use the Dutch rules in South Devon due to the meteorological data needed. Dew formation could be estimated using temperature and dew point data and cloudiness was based on one recording taken each day (Beaumont, 1947). Beaumont (1947) modified the rules

slightly so it was easier to forecast blight using the weather data available and found that the Dutch rules forecast blight prematurely. Relative humidity (RH) and temperature were enough to forecast the potential onset of late blight as a period of high humidity is normally followed by a drop in temperatures at night and thus induces dew formation (Beaumont, 1947). Adapting the Dutch rules, Beaumont (1947) stated that including a rule about RH would eliminate the need for most of the Dutch rules and suggested a temperature-humidity rule that had only two criteria; for 48 hours the minimum temperature must be 10°C or above and the RH must not drop below 75%. Although both the Dutch rules and the Beaumont Period provided similar levels of forecasting ability, the Beaumont Period provided a much simpler way of forecasting blight which worked well in West Cornwall and Devon.

Devised in the 1950s but first implemented in the 1970s (Taylor, 2003), the Smith Period was a re-working of the Beaumont Period. Smith (1956) studied blight outbreaks and weather patterns between 1950 and 1954 and using the Beaumont Period found that out of the 220 periods reported 43 were incorrect. Smith (1956) hypothesised that using the duration of high humidity rather than a level which it should not drop below, as in the Beaumont Period, would result in fewer incorrect forecasts. When examining the same weather data used for the Beaumont Period, Smith (1956) found that out of the 43 failed predictions of the Beaumont Period, 29 would have been classed as a 'full' Smith Periods if the humidity rule was what he proposed. In order for there to be a full Smith Period there must be two consecutive days where the minimum temperature is 10°C or above, and during the two days there must be 11 hours of 90% relative humidity (RH) on each day (Smith, 1956). A 'near miss' is when the RH criterion is achieved but the minimum temperature is under 10°C. Croxall and Smith (1976) examined the Smith Periods over the years 1923 to 1974 and found that in only one year, 1925, was there an outbreak before a Smith Period was reported. The

occurrence of the first Smith Period in these years was on average 21 days before the first blight outbreak was reported, so that warnings would have been given prematurely. If growers used the second reported Smith Period as a deciding factor as to when spray, there would have only been six premature warnings (Croxall and Smith, 1976), signifying that practicing the 'zero day' technique could be needed i.e. all Smith Periods reported before a certain day were disregarded as weather conditions were unlikely to lead to development of initial infections or would not lead to widespread blight. Taylor *et al*. (2002) tested the accuracy of the Smith Period during the years 1998 to 2002 using the weather data from 50 stations across England and Wales by generating maps showing when the first Smith Period in a particular area was recorded and then comparing these to the maps generated by reports of first confirmed blight outbreaks so that the number of days between the first blight warning and the first outbreak in the same area could be determined. The warnings were placed into three groups; too late was 10 days or less between the warning and the outbreak, 11 to 21 days was ideal and 21 days or over was too early. The Smith Period provided warnings that were either too early or ideal during seasons that were classed as severe and for one year (2001) that was classed as not severe. In the South West and Wales the Smith Period's accuracy was poorer than in other regions. Taylor *et al*. (2003) compared the accuracy of five different blight forecasting systems; the Smith Period, Negative Prognosis, Blitecast, Sparks model and NegFry. The Smith Period was discussed previously (Smith, 1956). The Negative Prognosis model uses temperature, humidity and rainfall to establish whether blight is a risk (Ulrich and Schrödter, 1966). Blitecast uses meteorological readings, such as hourly temperature, relative humidity and rainfall measurements, to produce severity values (Krause *et al*., 1975). A severity value is a number showing the risk of blight outbreak, anything above 18 is classified as a risk of blight (Gudmestad, 1997; Taylor *et al*., 2003). The Sparks model is based on spore

survival and uses measurements of temperature (maximum and minimum), relative humidity and rainfall (Taylor *et al*., 2003). NegFry uses a combination of the negative prognosis system and weather dependent calculations (Hansen *et al.,* 1995; Taylor *et al*., 2003). It was found that there was a great variability between the five systems. The Smith Period was the most reliable forecasting system providing protection against late blight even if the prediction of risk was incorrect. NegFry was the most accurate forecasting system correctly predicting late blight risk. The Smith Period became the most widely used forecasting system in the UK. Blight Watch is a late blight management tool that uses weather data from over 100 meteorological stations around the UK to calculate when a Smith Period is likely to occur (Cooke *et al.,* 2011). Weather stations can give hourly reports on weather data needed to generate Smith Period forecasts (Barrie and Bradshaw, 2001) and forecasts can be calculated for postcodes to give locally accurate blight warnings (Cooke *et al.,* 2011). On the 5th December 2002, the Potato Council launched the Fight against Blight campaign to provide information to growers thorough the UK on the blight epidemics so informed judgments on fungicides sprays could be made. The campaign involved producing best practice advice so growers could get information (Bradshaw *et al*., 2004). Voluntary blight scouts sample any potential late blight infection seen in commercial crops, allotments and gardens and send them to the laboratory for analysis. Incidents can be viewed online and daily alerts via emails and phone messages regarding blight incidents in the local area are available to warn that there disease present in the area (Cooke *et al.,* 2011).

Decision support systems (DSSs) are computer based management models that use knowledge about *P. infestans,* weather, fungicides, cultivar resistance, growth of the plant and disease pressure to help make decisions about the timings of fungicide applications (Forbes, 2004; Cooke *et al*., 2011). Within the UK, the main forecasting

system is the Smith Period but there are DSS models available upon subscription to provide localised information about a fungicide spray regime, for example Forecast Xtra and Plant-Plus (Hansen *et al*., 2012). Plant-Plus uses real time weather data to give a predictive disease forecast over a five-day period (Hinds and Mitchell, 1998). Forecast Xtra assesses the risk of blight for 3 days previous and 4 days ahead based on local or regional weather data and reports are sent out to farmers twice weekly (Hinds, 2006). Forecast Xtra is not used to inform growers when to spray, but to inform growers when spray regimes need to be amended and adjusted to suit the risk (Hinds, 2006).

1.11.4 Alternative methods of management

Organic potato growing produces highly variable yields depending on the season. Struik (2010) studied methods of late blight control for organic potato growers. Methods of control used by organic farmers are to spray the crops with copper-based fungicide (which are allowed for organic crops in some countries), destroying the plant at first sign of infection or using resistant cultivars. Speiser *et al*. (2006) suggested that resistant cultivars should be used when farming organically but stated that this would not stop the need for copper-based fungicides.

Using the landscape as a means to lessen the effect of blight on the crop could be an effective method of blight management. Monoculture of potato crops is convenient for the grower, but unfortunately it is also convenient for *P. infestans* as a large patch of genetically similar crops provide no extra resistance against the pathogen so the crop is destroyed quickly. Mixing susceptible and partially resistant cultivars can limit epidemics (Andrivon *et al*., 2003). The use of fungicides in combination with mixing cultivars reduces the epidemics further (Garrett *et al*., 2001). Garrett *et al*. (2001) found that reduction in the severity of the epidemics was variable in tropical climates and the greatest increase in yield was seen for the fields furthest away from commercial potato

fields. Recently, Skelsey *et al*., (2009) created a model that simulated growth and dispersal of *P. infestans* over a series of time points in a given area. Aggressiveness parameters (infection efficiency, lesion growth rate, latent period and sporulation capacity) for *P. infestans* were incorporated into the model. The model has a 2D grid that represents a potato field and each cell within the grid is a single plant. Once a lesion appears on a single plant (i.e. one cell in the 2D grid) a set area is added to that lesion every day. In the model the latent period is set to 5 days, the lesions sporulate and become infectious one day after the latent period and for only one day. All sporangia from infectious lesions are assumed to be released. The model had two rules to determine infection, firstly an infection hour is an hour where the temperature ranges between 10°C and 27°C with 90% RH and secondly, the amount of infection hours it takes to infect at different temperatures. Skelsey *et al.* (2010) went further by creating a model that looks at how crop heterogeneity affects epidemics. The model creates a landscape made up areas that are non-potato, so cannot be infected, and areas that are potatoes so those can be infected. The potato areas are split into two categories, those that are susceptible and those that are partially resistant to late blight. The model predicted that the composition of a field affects the spread of late blight. For example, mixing cultivars with different resistance ratings within the field is an effective method of slowing down epidemics, but is highly impractical for the grower (Struik, 2010). There is little improvement on the disease resistance and the amount of control is not as great as the control provided by fungicides so it would be more useful for organic farming (Andrivon *et al*., 2003; Philips *et al*., 2005). Changing the crop physiology and density had little impact on the management of blight (Philips *et al.,* 2005; Struik, 2010). Breeding is the best option for organic farming, but this takes time. A possible way of combating this is using genetically modified potato cultivars. A genetically modified organism (GMO) is an individual that has had a novel gene inserted into its

genome to provide desirable traits (Llaguno, 2001). R genes conferring resistance to late blight can be introduced into potato, but to be effective in the long-term the resistance must be durable and this has proved difficult due to the highly adaptable nature of *P. infestans*. New *P. infestans* isolates would evolve that could overcome the R-genes within the potato and would potentially spread rapidly through the population.

1.12 Summary

Potato late blight is a threat to the potato crop, especially with the 'new' population being more aggressive and potentially infecting at lower temperatures. Blight forecasting systems, for example the Smith Period, may need revaluating in conjunction with new parameters set by the more aggressive genotypes.

1.13 Aims

1. Examine the aggressiveness of contemporary *P. infestans* isolates.
2. Examine the effects of competition between *P. infestans* genotypes.
3. Examine the effects of temperature on mycelial growth, infection, lesion expansion and sporulation of contemporary *P. infestans* genotype.
4. Examine the effect of humidity on infection of four *P. infestans* genotypes.

Chapter 2 - Materials and Methods

2.1 Selection of *P. infestans* isolates

Fifty-seven contemporary *P. infestans* isolates were chosen from The James Hutton Institute *P. infestans* archive collection with several representative isolates per genotype. All isolates were collected from a range of potato cultivars from naturally occurring single lesion foliar/stem infections throughout the UK (and one German isolate, Bayer 9B) between the years 2006 and 2008. Mating types were tested by pairing the collected isolate with isolates with known mating types in Rye A agar plates and were examined to see if oospores were present. Genotypes were assigned by using 11 SSR markers in 3 multiplex PCR assays. Mating type and genotypes were assigned by Cooke *et al*. (2012a). Details of isolates can be seen in Table 2.1. Reference strains that were selected had been used in previous aggressiveness tests (Cooke *et al.*, 2012a).

2.2 Culture maintenance

All isolates were grown on Rye A agar in Petri dishes (9 cm) throughout the year and were incubated in the dark at 15°C. Two Rye A agar slopes in a plastic universal (Cat. No. Z645354-400EA, Sigma-Aldrich) for each isolate were archived for long-term storage. The Rye A agar was made with 60 g rough rye (Biodynamically grown Rye, Water Mill, Little Salkeld, Penrith, Cumbria), 20 g sucrose, 7.5 g agar (Difco select Agar) per litre with a concentration of 1.5% w.v. The rough rye was soaked in distilled water for 36 hours after which the excessive liquid was poured off and then sucrose was added to the rough rye. This mixture was blended and macerated for 5 seconds and then put in a water bath at 50°C for 3 hours. The mixture was then filtered through muslin. In preparation for experiments the isolates were subcultured onto Rye A agar in 9 cm Petri dishes by removing a small section of mycelium from the edge of an actively growing colony using a sterile scalpel.

2.3 Plant production

All cultivars were grown in a glasshouse set to 18°C, with nature light supplemented with artificial light to ensure 16 hours of daylight in 12 litre pots using compost containing sphagnum moss peat ($1m^3$), sand (10%), osmocote exact start (1.25kg), osmocote exact 6 month (3k), lime Ca and Mg (2.5kg each), celcote (1kg) and vermiculite (5%) (William Sinclair Holdings plc, Lincoln, UK). Tubers were stored in a cold room (4°C) and before planting the tubers were chitted for a week or until sprouting. Three tubers were placed in each plant pot and were watered regularly. Plants were grown for approximately five weeks and the leaves were removed for tests when the plants were in bud.

Table 2.1 – Details of *P. infestans* isolates used in the studies.

Isolate Name	Mating Type	Genotype	County	Region	Cultivar
88069	A1	A1 Misc	Unknown	Netherlands	Tomato
07_39	A2	13_A2_5	Unknown	Unknown	Unknown
07_SP12_3A	A1	A1 Misc	Highlands	Scotland	Russet Burbank
2006_3888A	A1	2_A1	Cheshire	England	Lady Rosetta
2006_3928A	A2	13_A2_1	Kent	England	King Edward
2006_3984C	A1	1_A1	Cambridgeshire	England	Maris Piper
2006_3996A	A1	A1 Misc	Kent	England	King Edward
2006_4012F	A2	3_A2	Cornwall	England	Charlotte
2006_4100A	A1	6_A1	Essex	England	Marfona
2006_4168B	A1	7_A1	Suffolk	England	Charlotte
2006_4232E	A1	8_A1	Pembrokeshire	Wales	Marfona
2006_4388C	A2	17_A2	Aberdeenshire	Scotland	Vivaldi
2006_4388D	A2	17_A2	Aberdeenshire	Scotland	Vivaldi
2006_4440C	A2	10_A2	Staffordshire	England	Maris Piper
2007_5054A	A1	A1 Misc	Kent	England	Maris Piper
2007_5074E	A2	3_A2	Gwynedd	Wales	Bintje
2007_5138G	A1	1_A1	Kent	England	Maris Piper
2007_5290C	A1	7_A1	Humberside	England	Pentland Dell
2007_5442F	A1	2_A1	East Lothian	Scotland	Maris Piper
2007_5482D	A2	10_A2	Cumbria	England	Morene
2007_5482E	A2	10_A2	Cumbria	England	Morene
2007_5622A	A1	2_A1	Gloucestershire	England	Pentland Crown
2007_5706E	A2	10_A2	Gwynedd	Wales	Estina
2007_5726C	A2	A2 Misc	Highlands	Scotland	Unknown
2007_5738B	A2	A2 Misc	Aberdeenshire	Scotland	Cara

Table 2.1 continued

Isolate Name	Mating Type	Genotype	County	Region	Cultivar
2007_5738E	A1	A1 Misc	Aberdeenshire	Scotland	Cara
2007_5738G	A1	A1 Misc	Aberdeenshire	Scotland	Cara
2007_5918A	A1	7_A1	Yorkshire	England	Unknown
2007_5974A	A1	A1 Misc	Kent	En gland	Tomato
Bayer_9B	A2	A2 Misc	Weser-Ems	Germany	Bintje
2008_6066A	A1	7_A1	Warwickshire	England	Markies
2008_6070E	A1	8_A1	Suffolk	England	Shepody
2008_6082F	A2	13_A2_5	Suffolk	England	Maris Piper
2008_6090A	A1	6_A1	Shropshire	England	Maris Piper
2008_6102A	A2	13_A2_2	Somerset	England	Maris Bard
2008_6194A	A2	13_A2_1	Cornwall	England	Maris Peer
2008_6222A	A1	8_A1	Aberdeenshire	Scotland	Unknown
2008_6250A	A2	13_A2_1	Staffordshire	England	Romano
2008_6274D	A1	7_A1	North Yorkshire	England	Nicola
2008_6306A	A1	6_A1	Shropshire	England	Vales Emerald
2008_6354C	A1	6_A1	Humberside	England	King Edward
2008_6394B	A1	A1 Misc	Gwynedd	Wales	Arran Victory
2008_6422F	A1	8_A1	Herefordshire	England	Russet Burbank
2008_6426A	A1	6_A1	Fife	Scotland	Saxon
2008_6430A	A2	13_A2_1	North Yorkshire	England	Maris Piper
2008_6446D	A2	A2 Misc	Dyfed	Wales	Wilja
2008_6446F	A1	A1 Misc	Dyfed	Wales	Wilja
2008_6458A	A1	8_A1	Northumberland	England	Unknown
2008_6498A	A1	6_A1	Oxfordshire	England	Sante
2008_6502A	A1	6_A1	North Yorkshire	England	Morene
2008_6530C	A2	13_A2_2	Aberdeenshire	Scotland	King Edward

Table 2.1 continued

Isolate Name	Mating Type	Genotype	County	Region	Cultivar
2008_6610E	A1	6_A1	Shropshire	England	Estima
2008_6850D	A1	2_A1	Angus	Scotland	Cultra
2008_7006D	A1	2_A1	East Lothian	Scotland	Unknown
2008_7034E	A1	6_A1	West Midlands	England	Markies
2008_7038A	A2	13_A2_1	Staffordshire	England	Pentland Dell
T30-4	A1	A1 Misc	Unknown	Unknown	Unknown

2.4 Inoculum production

Petri dishes containing two week old cultures of each isolate grown on Rye A agar had 10 ml of sterile distilled water poured into them. Using a glass pipette, sporangia were scraped off the agar and the suspension was transferred to a 100 ml beaker. The concentration of the suspension was checked under the microscope. The number of sporangia present was counted using a McMaster worm egg counter and diluted to achieve a sporangial concentration of 1.4×10^4. The suspension was then chilled at 4°C for 3 hours to promote zoospore release. Plastic boxes with sealable lids (39.5 x 25.5 x 8.8 cm) were lined with moist absorbent paper towelling. Placed on top of the paper, abaxial side up, were 10 to 15 leaflets from greenhouse-grown plants of a susceptible cultivar (Craig's Royal or Maris Piper). After chilling, the suspension was checked microscopically for the presence of zoospores and using a glass pipette droplets were placed onto the leaflets. The plastic boxes were then put into plastic bags to maintain high humidity and incubated in a temperature controlled glasshouse at 15°C.

To passage the isolates from infected to healthy leaflets, the infected leaflet with sporulating lesions was dipped into a 100 ml beaker containing approximately 10 ml of cold sterile distilled water. The leaflet was stirred in the water to allow the sporangia to fall off and then removed. Sporangial numbers were counted under the microscope using the same method stated above as well as the process of chilling, zoospore checking and inoculation of leaflets. Sporangial suspensions for experiments were produced following the same procedure as above, but isolates were passaged at least twice and after the suspension was made and counted it was diluted to 1.4×10^4 sporangia per ml.

Inoculum for experiments was made using seven day old sporulating lesions on detached Craig's Royal (potato cultivar Up-to-Date in Northern Ireland) leaflets. For

detached leaflet assays, the leaflets were each inoculated with a 15 µl droplet of sporangial suspension, being sure the suspension was placed near the midrib of the leaflet on the abaxial surface and in the same position on each leaflet.

In Northern Ireland, inoculum was made by using a small paint brush dipped in ethanol and thoroughly washed in sterile distilled water before being used to brush spores off the detached leaflet into a small Petri dish containing sterile distilled water. Using a haemocytometer the numbers of sporangia were counted and the concentrations calculated. The suspensions were then chilled for 3 hours to promote zoospore dispersal. After chilling, the inoculum was checked for the presence of zoospores and then diluted to a concentration of $1x10^4$ sporangia per ml (method for Chapter 4).

2.5 Field and whole plant blight assessment

The ADAS blight assessment key (Anon, 1976) was used to assess the percentage foliar blight in both field trials and on whole plants (Table 2.2). The key was adjusted to assess single plants rather than the whole plot. For example, first establishment was recorded as 0.01%, 2 to 5 lesions for 0.1%, 5 to 10 lesions for 1% and the rest of the values were scored as percentage blight seen on a single plant rather than a whole plot.

Table 2.2 - Percentage foliar blight assessment key created by ADAS (Anon, 1976) with the addition of the 0.01% and 10% categories.

Agricultural Development and Advisory Service (ADAS) manual of growth stage and disease assessment keys, 1976	
% Foliar blight	Assessment
0	Not seen
0.01	5 leaflets/ 10 plants or 2 leaves/ 10 plants with lesions
0.1	Only a few plants affected; up to 1 or 2 spots on 12 yard radius
1	Up to 10 spots per plant
5	About 50 spots per plant or one leaflet in 10 attacked
10	2-5 leaflets in 19 with lesions
25	Nearly every leaflet with lesion, plant still retaining normal form: field may smell of blight but looks green although every plant is infected
50	Every plant affected and about ½ of leaf area destroyed by blight: field looks green flecked with brown
75	About ¼ of leaf area destroyed field looks neither predominantly brown nor green. In some varieties the youngest leaves escape infections so that green is more conspicuous than in varieties like King Edward, which commonly shows severe shoot infection
95	Only a few leaves are left green but stems are green
100	All leaves are dead, stems dead or dying

2.6 Field trial sampling

Leaflets with single lesions were collected for sampling on scoring days. Up to four samples from each plant were taken depending on the number of lesions present. Lesions were rubbed onto a Whatman FTA card (Whatman FTA^{TM} Classic Card, Cat No. WB120205, GE Healthcare UK Limited, method described in Chapter 2.6) for storage according to the manufacturer's instructions until they were genotyped using SSR analysis (Lees *et al*., 2006; as described in Chapter 2.7). Whatman FTA cards were used to collect the DNA of the *P. infestans* genotypes that infected the plants in the field trial (genotype 13_A2, 6_A1, 7_A1 and 8_A1). The lesion, preferably sporulating, was pressed within the circle of the FTA card containing a chemical that lysed cell membranes and trapped the DNA within the fibres of the card. Cards were stored at room temperature. The cards were processed to allow the release of the DNA by punching out a disc of the dried sample using a 2.0 Harris micro punch (Whatman micro punch Harris 2.0 mm tool, Cat. No. WB100007, GE Healthcare UK Limited). The disc was placed into a 0.2 ml PCR amplification tube of a 96 well microplate (Cat. No. 321-21-051, Axygen, Inc.) and 150 µl of Whatman FTA purification reagent (Cat. No. WB120204, GE Healthcare UK Limited) was added to the PCR tube with the disc in. It was vortexed for 5 minutes at room temperature after which all reagents were removed and the addition of the purification reagent, vortexing and liquid removal was repeated twice over. After this, 150 µl of TE buffer was added to the PCR tube (10 mM Tris-HCL, 0.1 mM EDTA, pH 8.0), it was left for 5 minutes at room temperature, all the buffer was removed and this was repeated twice over. The lid was left open for 1 hour to dry the disc, making the samples ready for genotyping.

2.7 Genotyping

The protocol used for genotyping followed that of Li *et al*., (2013). All steps were done on ice. Twelve SSRs, both forward and reverse primer (Table 2.3), were used in a multiplex PCR reaction using a QIAGEN Type-it Microsatellite PCR Kit (QIAGEN, Cat. No. 206243). The primer mix comprised of 1.25 µl of SSR3, SSR11, SSR4, SSR6, Pi63 and SSR2 primers, 2.5 µl of PiG11, 1.0 µl of Pi70, 6.0 µl of Pi48 and SSR8, 403 µl of water, 625 µl of QIAGEN type-it Multiplex PCR mix, 40 µl of D13 and 10 µl of Pi04. Both forward and reverse forms of each primer were added to the mix. The primer mix was made in an amber micro tube so the daylight did not degrade the fluorescent labels in the primer (Table 2.3) and pipetted into each of the tubes on the PCR microplate (11.5 µl per tube) containing the DNA and the plate covered with an AxyMat^{TM} silicone sealing lid (Cat. No. 521-01-151, Axygen Inc.). The PCR micro plate was placed in an MWG Biotech Primus 96 Plus PCR machine (MWG Biotech AG, Penzberg, Germany) and subjected to a PCR programme of 95°C for 5 minutes, then 33 cycles of 95°C for 30 seconds, 58°C for 90 seconds, 72°C for 20 seconds and finally 60°C for 30 minutes. The PCR product was diluted to a 1:20 ratio with UV treated water into a 96 well PCR micro plate. An aliquot (1 ml) of HiDi Formamide (Applied Biosystems) was added to 6 µl of Applied Biosystems Liz500 size standard and mixed, then 10.2 µl of this mix was pipetted into a fresh PCR plate (Abgene AB0600) and 0.6 µl of the diluted PCR product added. The plate was loaded into an ABI Prism 377 DNA sequencer which was run according to the instructions stated by the manufacturers (Applied Biosciences). Once run, the data collected was loaded into Genemapper software where peak sizes were generated and analysed to categories the samples into genotypes (Lees *et al*., 2006; Li *et al*., 2013).

2.8 Data analysis

All data were analysed using GenStat for Windows (13th Edition). Analysis of variance (ANOVA) was used to assess the variation between the means of the different Incubation Period (IP) Latent Period (LP) and Lesion Sizes (LS) of the genotypes and isolates. For detached leaflet assays, leaflets were assessed daily for IP, LP and LS; IP and LP were assessed for the first signs of infection and sporulation by the naked eye and lesion size was measured in 2 directions at 90 degrees with digital callipers (0-6”/150 mm digimatic calipers, Mitutoyo UK Ltd). For the field trial, plots were monitored daily until the first signs of infection were found on the inoculated plants and then disease assessments for each plant took place every 3 to 5 days using the ADAS blight assessment key (Anon, 1976; described in Chapter 2.5).

Area under the lesion expansion (AULEC, Chapter 3) and area under the disease progression curve (AUDPC, Chapter 4) was calculated by adding together all the disease scores (either LS or percentage blight present) over the experiment to create a value that represents the cumulative total of the recorded data (Carlisle *et al*., 2002).

Table 2.3 – Primer sequences for markers deployed in SSR genotype analysis. Sequences show the fluorescent tag added to the primer. Primers tagged with FAM were synthesised by Sigma-Aldrich, Poole, UK and those tagged with NED, VIC and PET were synthesised by Applied Bio-systems, Paisley, UK. Concentration in final reaction is shown. Product size taken from Li *et al*., (2013)

Primers	Product Size (bp)	Sequence – 5'-3'	Final Conc. (µm)
SSR3_Fwd_NED	225-275	NED-ACTTGCAGAACTACCGCCC	0.0455
SSR3_Rev_PT	225-275	GTTTGACCACTTTCCTCGGTTC	0.0455
SSR11_Fwd_NED	325-360	NED-TTAAGCCACGACATGAGCTG	0.0455
SSR11_Rev_PT	325-360	GTTTAGACAATTGTTTTGTGGTCGC	0.0455
SSR4_Fwd_FAM	280-305	FAM-TCTTGTTCGAGTATGCGACG	0.0455
SSR4_Rev_PT	280-305	GTTTCACTTCGGGAGAAAGGCTTC	0.0455
SSR6_Fwd_PT	230-250	GTTTTGGTGGGCTGAAGTTTT	0.0455
SSR6_Rev_VIC	230-250	VIC-TCGCCACAAGATTTATTCCG	0.0455
Pi63_Fwd_VIC	265-280	VIC-ATGACGAAGATGAAAGTGAGG	0.0455
PI63-REV_long	265-280	CGTATTTTCCTGTTTATCTAACACC	0.0455
SSR2_Fwd_PET	165-180	PET-CGACTTCTACATCAACCGGC	0.0455
SSR2_Rev_PT	165-180	GTTTCAATCTGCAGCCGTAAGA	0.0455
PiG11_Fwd_NED	130-180	NED-TGCTATTTATCAAGCGTGGG	0.0909
PiG11_Rev_PT	130-180	GTTTCAATCTGCAGCCGTAAGA	0.0909
Pi70_Fwd_VIC	185-205	VIC-ATGAAAATACGTCAATGCTCG	0.0364
Pi70_Rev	185-205	CGTTGGATATTTCTATTTCTTCG	0.0364
Pi4b_Fwd_PET	200-295	PET-AAAATAAAGCCTTTGGTTCA	0.2400

Table 2.3 continued

Primer	Product size (bp)	Sequence - 5’ to 3’	Final Conc. (µm)
Pi4B_Rev	200-295	GCTTGGATATTTCTATTTCTTCG	0.2400
SSR8_Fwd_FAM	250-275	FAM-AATCTGATCGCAACTGAGGG	0.2400
SRR8_Rev_PT	250-275	GTTTACAAGATACACAGTCGCTCC	0.2400
D13_Fwd	100-185	FAM-TGCCCCCTGCTCACTC	0.1600
D13_Rev_long	100-185	GCTCGAATTCATTTTACAGACTTG	0.1600
Pi04_Fwd_VIC	160-175	VIC-AAGCGGCTTACCGATGG	0.0376
Pi04_Rev_PT	160-175	GTTTCAGCGGCTGTTTCGAC	0.0376

Chapter 3 - Aggressiveness of contemporary *P. infestans* isolates

3.1 Introduction

3.1.1 Aggressiveness methodology

Empirical measurements are used to define the aggressiveness of a given *P. infestans* isolate. Typical measurements include incubation period, latent period and lesion size (Pariaud *et al*., 2009). Pariaud *et al*. (2009) defined the incubation period (IP) as the time between inoculation and the first visual signs of infection and latent period (LP) as the time between inoculation and sporulation. Lesion size (LS) is the area of necrotic tissue caused by the pathogen; this is recorded by measuring the diameter of the lesion over several days post inoculation (Cooke *et al*., 2007). An aggressive isolate will produce a larger lesion compared to a less aggressive isolate within the same time period. The development of a lesion over time can be expressed as the area under the lesion expansion curve (AULEC; Carlisle *et al*., 2002). Carlisle *et al*. (2002) discussed the benefits of using detached leaflet experiments to test aggressiveness. The detached leaflet approach has many advantages over whole plant or field tests which are other assays that can be used to test aggressiveness. The detached leaflet assay allows testing of more isolates at one given time, more stringent control over environmental factors and avoids the problems of contamination interference between plots within a field trial. Isolates which cannot be tested in the field can be tested with the detached leaflet assay. Field testing studies for aggressiveness are usually only used for a small set of isolates that are native to that region (Cooke *et al*., 2007). However, differences have been found between methods. Miller *et al*. (1998) found that there was a significant difference between the sporulation capacities of isolates when using whole plant tests, but no difference was found in sporulation capacity using detached leaflet assays. However, Singh and Birhman (1994) found that the AUDPC (area under the disease progression curve) values from field studies and the laboratory test results for LS, LP

and spore density (SD) were correlated and gave similar trends. Ideally, both laboratory and field methods would be used but this is only practical for small sets of isolates. In order to test the aggressiveness of *P. infestans*, more than one potato cultivar should be used, and preferably a selection of cultivars with different levels of late blight resistance. This is because the use of one cultivar can give misleading results due to differences in host resistance between cultivars (Cooke *et al*., 2007); use of multiple cultivars is more easily achieved using detached leaflets than whole plants or field tests. Comparing the aggressiveness of *P. infestans* isolates collected at different times is also complicated by the effects of repeated sub-culturing or long-term storage. Storing isolates in liquid nitrogen and agar slopes can be detrimental to their aggressiveness. Day and Shattock (1997) found that isolates from 1993 which were recovered from liquid nitrogen had a lower fitness index than ones from more recent years. For detached leaflet assays this is combated by inoculating the isolates onto detached leaflets of a cultivar with no R genes and passaging them to another set of detached leaflets at least twice (Cooke *et al*., 2007).

The methodology of this study was based on the aggressiveness test conducted by Cooke *et al.* (2012a) (discussed in Chapter 3.1.2). The standard isolate method was implemented so that the structure of the experiment would be similar to the method it was based on, and because the numbers of isolates to be tested prevented them all being included in one experiment. Using standard isolates in each replication allows the variation between the tests to be examined; this is a statistically valid method of testing a large collection of isolates that cannot be tested at once.

3.1.2 Genotype 13_A2

Genotype 13_A2 was discussed in Chapter 1.9.1. Cooke *et al*. (2012a) used a detached leaflet assay to test the aggressiveness of 26 *P. infestans* isolates at 13°C and 18°C on

five potato cultivars. The isolates were chosen to represent the nine main genotypes found in the UK populations (18 isolates from 2006, 2 from 2005, 1 from 2004, 2 from 2003, 1 from 1996, 1 from 1995 and 1 isolate had an unspecified year); four isolates were chosen to represent 13_A2 and two isolates for each of the other genotypes. Seven European isolates were also included; isolates from the Netherlands were also genotype 13_A2 whereas those from Sweden and Poland did not fit into the genotypes of the UK population. Two aggressiveness criteria were recorded; lesion size and latent period. On average, genotype 13_A2 caused larger lesions than other genotypes at 13°C. At 18°C, genotype 13_A2 was less aggressive than the other genotypes. Overall, 6_A1 caused the largest lesions, followed by genotype 13_A2 and then genotype 17_A2 (which was represented by one isolate). Mean lesion sizes for genotype 13_A2 and genotype 6_A1 were not significantly different at 13°C but genotype 13_A2 was represented by six isolates whereas genotype 6_A1 was only represented by two isolates. For LP at 13°C, genotype 13_A2 had some of the lowest values; all six isolates ranked within the eight shortest LP values, with the other two isolates with short LP values representing genotypes 6_A1 and 17_A2. As with lesion size, this effect was less pronounced at 18°C, but all six 13_A2 isolates were in the top 12 isolates with the shortest LP values.

3.1.3 Aim

The aim of this study was to investigate the differences in aggressiveness between isolates of *P. infestans* genotypes that are common to the current UK population. This was done by measuring three aggressiveness criteria; IP, LP and LS. The effect of potato cultivar upon aggressiveness was taken into consideration by using five commonly grown potato cultivars with differing levels of foliar resistance to late blight to test each isolate; Maris Piper, Cara, Estima, Lady Balfour and King Edward have resistance ratings for foliar blight of 4, 5, 4, 4 and 3 respectively (resistance ratings

discussed in Chapter 1.6.1). Potato cultivars were chosen to match the popular commercial potato cultivars grown in the UK and the previous aggressiveness test discussed in Chapter 3.1.2.

3.2 Method

A detached leaflet assay was used to test the aggressiveness of 49 isolates between June and October 2010 on five potato cultivars. Isolates chosen were passaged through the potato cultivar Craig's Royal (susceptible and lacking all known R-genes) twice before being tested (as in Chapter 2.4).

3.2.1 Design

Damp tissue was used to line 30 clear plastic boxes and each box (39.5 x 25.5 x 8.8 cm) had a sealable lid so that humidity was maintained. All potato cultivars were grown as described in Chapter 2.3. For each experiment, detached leaflets of a similar age, size and from a similar position in the plant (mid plant) of each potato cultivar were placed abaxial side up into the lined box. One leaflet per isolate was used in each test; Test 1 had 17 isolates, Test 2 had 7, Test 3 had 20 and Test 4 had 12 isolates (Table 3.1). At each date, two standard isolates, 2006_3928A (genotype 13_A2) and 2007_5442F (genotype 2_A1), were included to assess the variation between tests. A fully randomised block design was used in every experiment with six replicate blocks each containing one box of leaflets of each potato cultivar. Isolates were randomised within boxes except for the two standard isolates which were always in the centre of the box in each experiment. Details of isolates can be found in Chapter 2, Table 2.1.

Table 3.1 – Isolates used in each aggressiveness test

Test	Genotype	Isolates used in test	No. isolates per genotype
Test 1	1_A1	2006_3984C	1
	2_A1	2007_5442F, 2008_7006D	2
	3_A2	2007_5074E	1
	6_A1	2008_6090A, 2008_6354C, 2008_6426A, 2008_6502A	4
	8_A1	2008_6070E	1
	13_A2	2006_3928A, 2008_6050B, 2008_6102A, 2008_6194A, 2008_7038A	5
	A1 Misc	2008_6394B	1
	A2 Misc	2007_5726C, Bayer 9B	2
Test 2	2_A1	2007_5442F	1
	6_A1	2008_6306A	1
	8_A1	2006_4232E	1
	13_A2	2006_3928A	1
	A1 Misc	88069, 2007_5054A, 2008_6446F	3
Test 3	1_A1	2007_5138G	1
	2_A1	2007_5442F, 2007_5622A, 2008_6850D	3
	3_A2	2006_4102F	1
	6_A1	2006_4100A, 2008_6090A, 2008_6610E	3
	7_A1	2006_4168B, 2007_5290C, 2008_6274D	3
	8_A1	2008_6222A, 2008_6422F	2
	10_A2	2007_5482D, 2007_5482E	2
	13_A2	2006_3928A, 07_39, 2008_6430A, 2008_6530C	4
	A1 Misc	2007_5738G	1
Test 4	2_A1	2007_5442F	1
	6_A1	2008_6090A, 2008_6498A	2
	7_A1	2007_5918A, 2008_6066A	2
	8_A1	2008_6458E	1
	10_A2	2006_4440C	1
	13_A2	2006_3928A, 2008_6082F, 2008_6250D	3
	17_A2	2006_4388C, 2006_4388D	2
	A1 Misc	2007_5974A	1

3.2.2 Inoculation and incubation

Inoculum production and inoculation was as described in Chapter 2.4. The boxes were placed into clear plastic bags and transferred to a growth chamber (Reftech walk-in growth chamber) set to 15°C with a light and dark cycle of 16 and 8 hours, respectively. The boxes were covered with tissue paper for the first 24 hours.

3.2.3 Data collection and analysis

Methods of assessing IP, LP and LS were described in Chapter 2.8. The area under the lesion expansion curve (AULEC, calculation shown in Chapter 2.8) was analysed as this looks at the lesion expansion over time, in this case seven days. All genotypes had a different number of isolates representing them in each test; this was taken into account by examining all the differences in variation between each genotype, conducting a general ANOVA to provide the standard error for every combination. The statistical methods are described in Chapter 2.8.

3.3 Results

3.3.1 Standard isolates

An ANOVA was conducted on the AULEC values of the two standard isolates included in each of the four tests to compare the variation. The AULEC values for both of the standard isolates were significantly different between tests ($P<0.001$, Table 3.2). Test 1 and Test 3 were significantly different from all other tests and from each other. Test 2 and Test 4 were not significantly different from one another but had significantly larger AULEC values than Test 1 and Test 3 (Figure 3.1.A). When looking at the interactions between the tests and isolates, it was seen that for isolate 2006_3928A there was no difference in the mean AULEC values for Test 1, 2 and 3 but Test 4 had a significantly larger AULEC value. Isolate 2007_5442F showed more variation between tests with Test 2 and Test 3 AULEC values being significantly different from all others and from each other. Test 1 and 4 were not significantly different from one another but were different from the other two tests. This variation was too large to allow isolates to be compared between experiments and therefore the results for each experiment are presented separately.

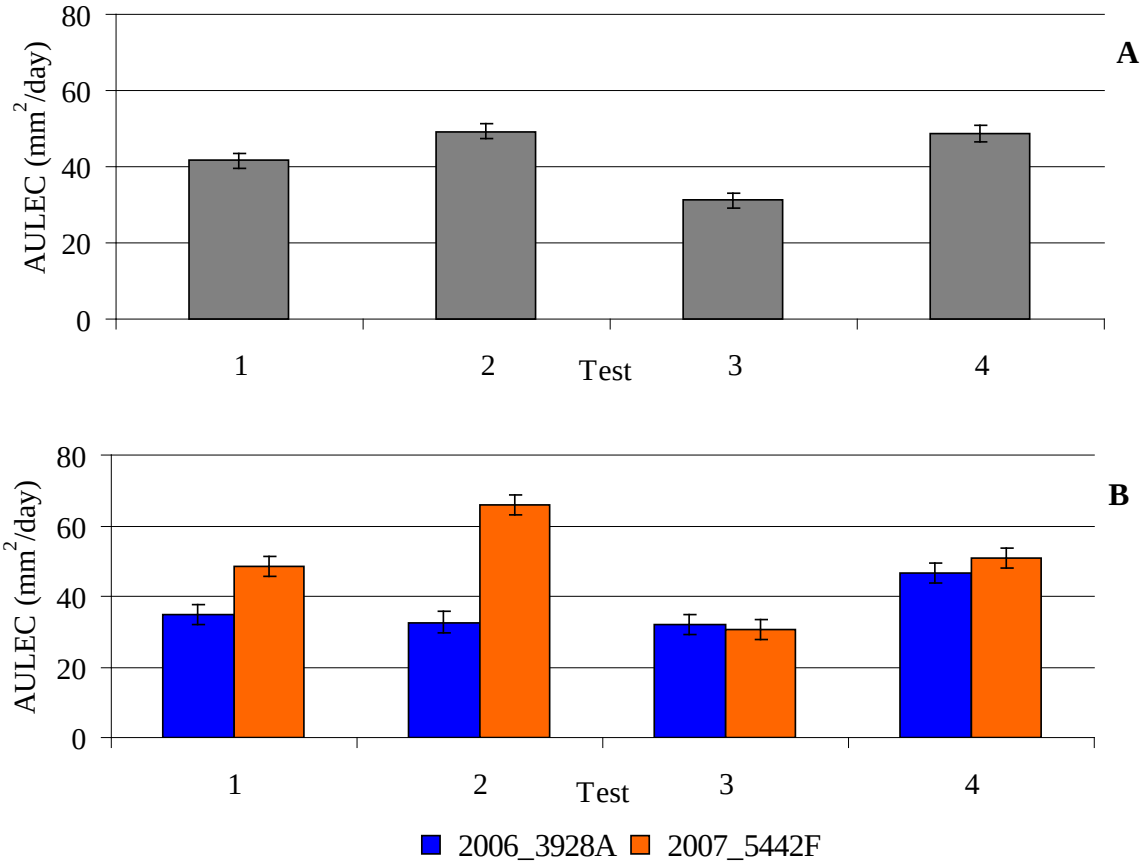


Figure 3.1 – Mean AULEC values for UK *P. infestans* isolates used as standard isolates for four aggressiveness tests on five potato cultivars. The errors bars represents the standard errors of differences of the means (SE).

A) AULEC values for each test (meaned over isolates) SE=2.839

B) AULEC values for each isolate and test. SE=2.008

Table 3.2 – Descriptive statistics for the variation between the AULEC values of the two standard isolates used in the aggressiveness test on five potato cultivars

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
Test	3	127341	4244.7	35.11	<.001
Isolate	1	9147.7	9147.7	75.66	<.001
Test x Isolate	3	10334.9	3445	28.49	<.001

3.3.2 Incubation period (IP)

3.3.2.1 Isolate

There was a significant difference between the responses of the isolates for IP values in all tests ($P<0.001$; Figure 3.2, Table 3.3). The same isolate never consistently had the shortest IP over all of the tests. Isolates belonging to a particular genotype showed variation for IP, but this was not the case for all genotypes in all tests. For genotype 13_A2, variation between isolates was seen in Test 1 and 3 and no variation between the isolates was found in Tests 2 and 4; Test 2 only contained one isolate representing genotype 13_A2. In Test 1, isolates representing genotype 13_A2 had a range of 3.1 days to 3.8 days with the shortest IP (2008_6012A) that was significantly shorter than the others and the longest IP (2008_6194A) being significantly longer. In Test 3, genotype 13_A2 had four isolate representatives, 2006_3928A (3.1 days), 2008_6430A (3.5 days), 07_39 (3.6 days) and 2008_6530C (4.4 days). Of these, 2006_3928A had a significantly shorter IP and the IP of 2008_6530C was significantly longer, while 2008_6430A and 07_39 were not significantly different from one another. Similar trends could be seen for genotype 6_A1, this time showing that there was no variation between the isolates of genotype 6_A1 for Test 1 and Test 2 but variation was seen in Test 3 and Test 4. In Test 3, there were three isolates of genotype 6_A1 which showed a large range of IP; 2008_6090A had an IP of 3.1 days, 2008_6610E had an IP of 3.3 days and 2006_4100A an IP of 4.7 days and they were significantly different to one another. In Test 4, the two isolates representing genotype 6_A1 had a difference of 0.2 days between them, but the difference was still significant.

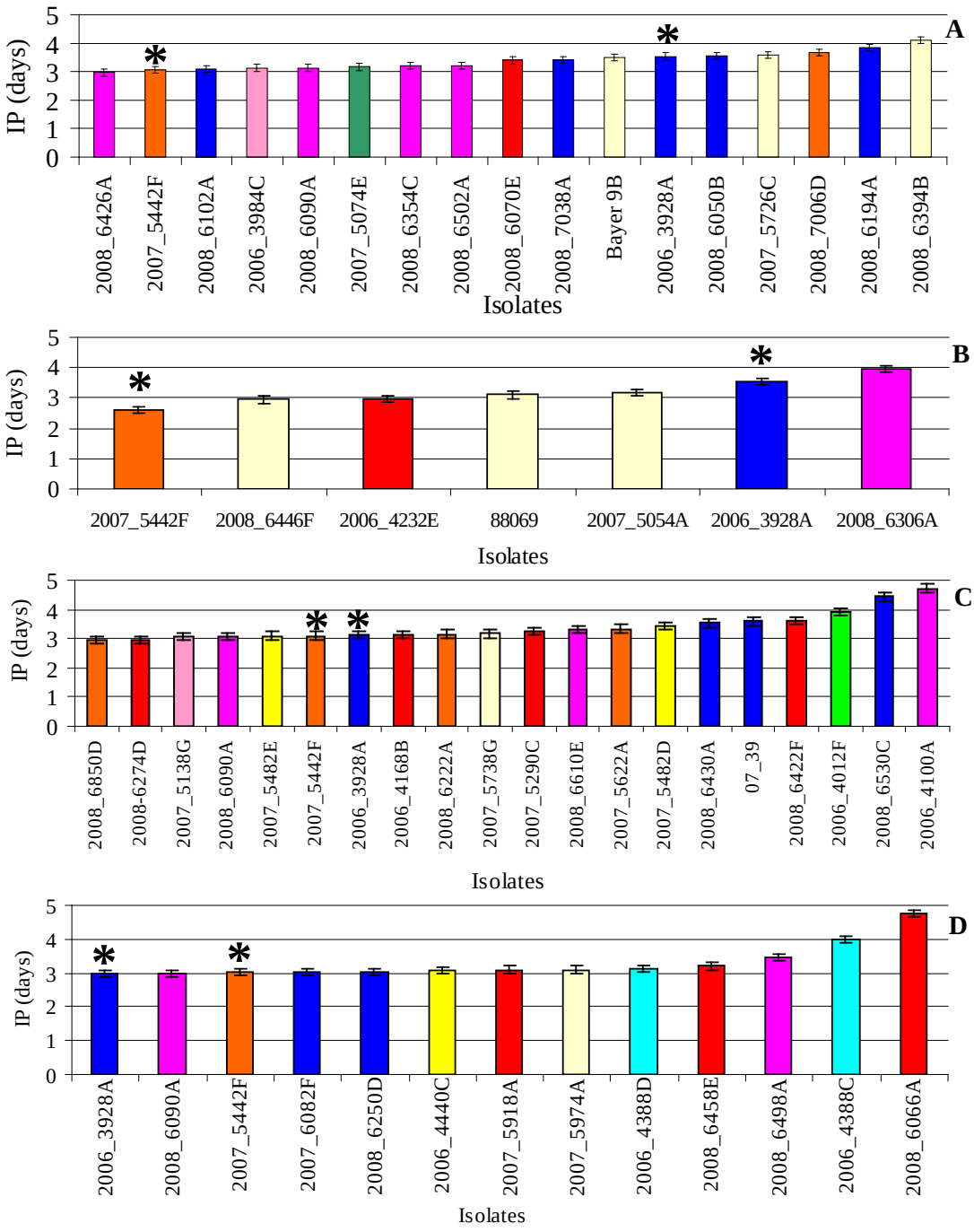


Figure 3.2 – Mean incubation period (IP) values for a range of UK *Phytophthora infestans* isolates on detached potato leaflets (mean of cultivar). The error bars represent the standard errors of differences of the means (SE).

A) Test 1 (SE=0.12) B) Test 2 (SE=0.12) C) Test 3 (SE=0.13) D) Test 4 (SE=0.10)

*= Reference isolates

1_A1 2_A1 3_A2 6_A1 7_A1 8_A1 10_A2 13_A2 17_A2 A1Misc A2 Misc

3.3.2.2 Genotype

There was a significant difference between the responses of the genotypes for IP values in all tests ($P<0.001$, Figure 3.3, Table 3.3). The range for IP across all tests was 2 to 5 days and the range of the IP values differed for each test; 3.0 to 4.1 days (Test 1), 2.6 to 4.0 days (Test 2), 3.0 to 4.7 days (Test 3) and 3.0 to 4.8 days (Test 4). Genotypes did not have the same IP values across the tests. Genotype 13_A2 did not have a significantly shorter IP in Tests 1, 2 and 3 when comparing it to the other genotypes in each test (3.5, 3.6, 3.5 days, respectively), but in Test 4 it had one of the shortest IP values (3.0 days) although in this test all genotypes had IP values between 3.0–3.3 days. In Tests 1 and 3, genotype 13_A2 had a significantly longer IP than genotype 6_A1 but in Tests 2 and 4 genotype 6_A1 had a significantly longer IP than genotype 13_A2. Within the variants of genotype 13_A2 there was no variant that had clearly a shorter IP. Genotype 6_A1 had a range of IP values from 3.0-3.9 days and was not consistent in its ranking within the tests; in Tests 1 and 4 6_A1 had the shortest IP when comparing it to other genotypes within that particular test, but in Test 2 and 4, the IP value was of an intermediate to low range. Genotype 8_A1 was present in all tests and in Tests 1, 3 and 4 the ranking of the genotype was consistently intermediate in value. In Test 2, it had the second shortest IP. Genotype 7_A1 was present in two tests and the rankings within those tests differed greatly but the IP values were similar.

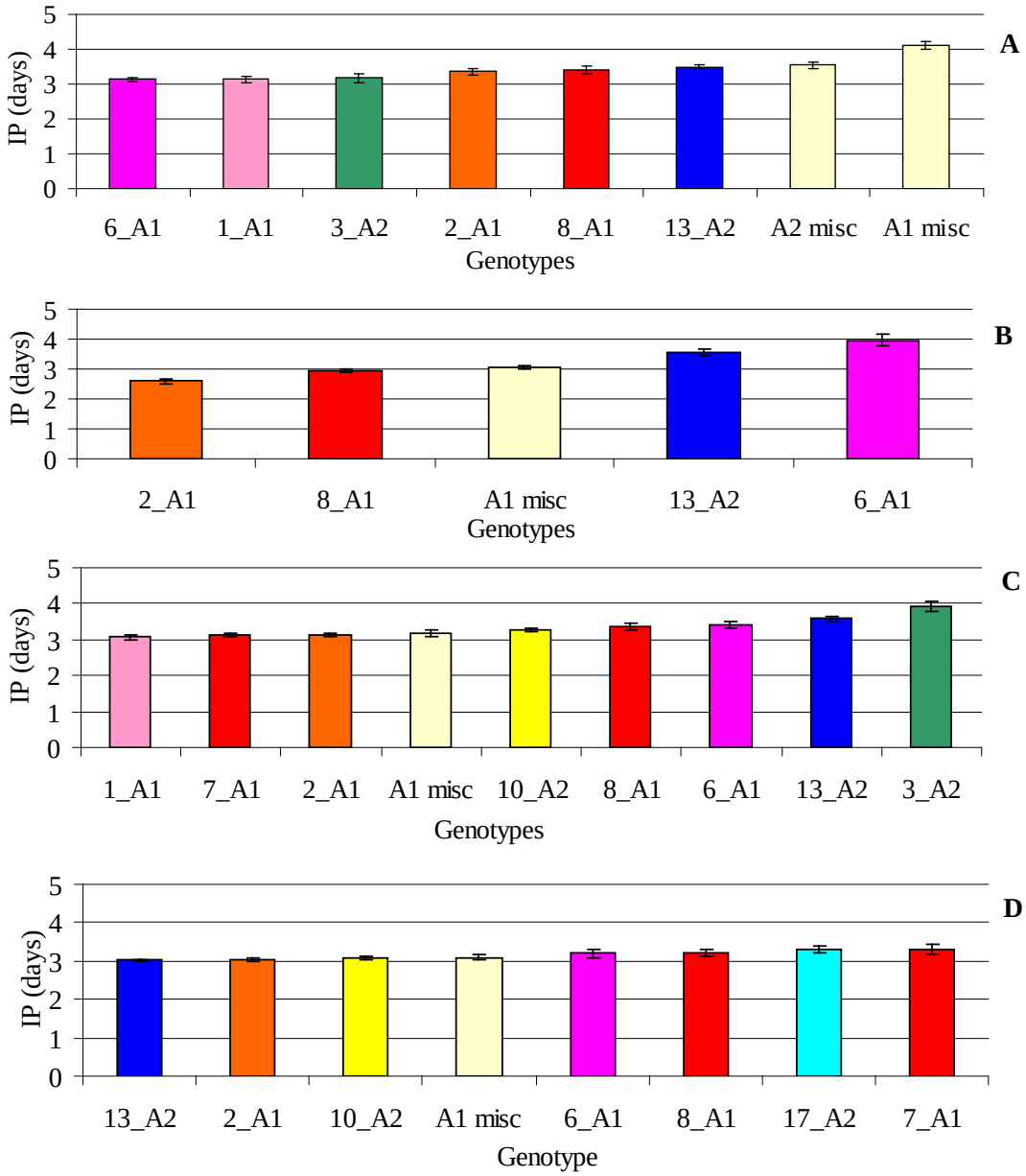


Figure 3.3 – Mean incubation period (IP) values for a range of UK *Phytophthora infestans* genotypes on detached potato leaflets (mean of cultivar). The error bars represent the standard errors of differences of the means (SE). Number of isolates representing each genotype is shown after the SE value.

A) Test 1 SE = 6_A1 0.05 (n=4), 1_A1 0.09 (n=1), 3_A2 0.12 (n=1), 2_A1 0.09 (n=2), 8_A1 0.10 (n=1), 13_A2 0.05 (n=5), A2 Misc 0.08 (n=2) and A1 Misc 0.11 (n=1)

B) Test 2 SE = 2_A1 0.09 (n=1), 8_A1 0.06 (n=1), A1 Misc 0.05 (n=3), 13_A2 0.10 (n=1) and 6_A1 0.18 (n=1)

C) Test 3 SE = 1_A1 0.07 (n=1), 7_A1 0.05 (n=3), 2_A2 0.05 (n=3), A1 Misc 0.08 (n=1), 10_A2 0.06 (n=2), 8_A1 0.09 (n=2), 6_A1 0.09 (n=3), 13_A2 0.06 (n=4), 3_A2 0.14 (n=1)

D) Test 4 SE = 13_A2 0.02 (n=3), 2_A1 0.03 (n=1), 10_A2 0.05 (n=1), A1 Misc 0.06 (n=1), 6_A1 0.10 (n=2), 8_A1 0.09 (n=1), 17_A2 0.08 (n=2) and 7_A1 0.13 (n=2)

3.3.2.3 Cultivar

Cultivar did not significantly affect IP in Tests 1, 3 and 4 (Figure 3.4, Table 3.3) and in Test 2, there were significant differences in IP values between cultivars (P=0.01, Figure 3.4.B, Table 3.3) and a significant interaction between genotype and cultivar (Table 3.3) as King Edward proved significantly more resistant to infection than the other cultivars. This was due to one isolate which infected King Edward considerably more slowly (IP 4.5 days). Genotype 6_A1 (represented by one isolate; 2008_6306A) had a significantly longer IP value for most cultivars except on Lady Balfour where both genotypes 6_A1 and 13_A2 were significantly different from the other genotypes. The IP values of genotype 6_A1 (2008_6306A) for the other cultivars apart from Lady Balfour ranged from 3.5-4.3 days with the 4.3 day IP value being for Cara, which is the most resistant so a longer IP was expected. This isolate took the longest to infect all cultivars when comparing it to the isolates that were present in Test 2, so although it was surprising to see an IP value as long as 4.5 days for King Edward, it did behave the same for the other cultivars. When comparing the IP value for genotype 6_A1 on all cultivars the values for King Edward, Cara and Estima are significantly longer than those on Maris Piper and Lady Balfour. Both genotype 13_A2 and 8_A1 showed no significant differences in IP for each cultivar. For genotype 2_A1 and A1 Misc there was no significant effect of cultivar, except for Cara on which both genotypes had significantly shorter IP values. Interactions between isolate and cultivar were seen for IP in Tests 2, 3 and 4 but not for Test 1 (data shown in Appendix ii). In most cases isolates infecting Maris Piper had a longer IP value than when infecting Cara, for example isolate 2006_3928A had an IP value of 3.3 on Maris Piper and 3.0 on Cara and was significantly different. Conversely, isolate 2008_6430C had a shorter IP on Maris Piper than it did when infecting Cara.

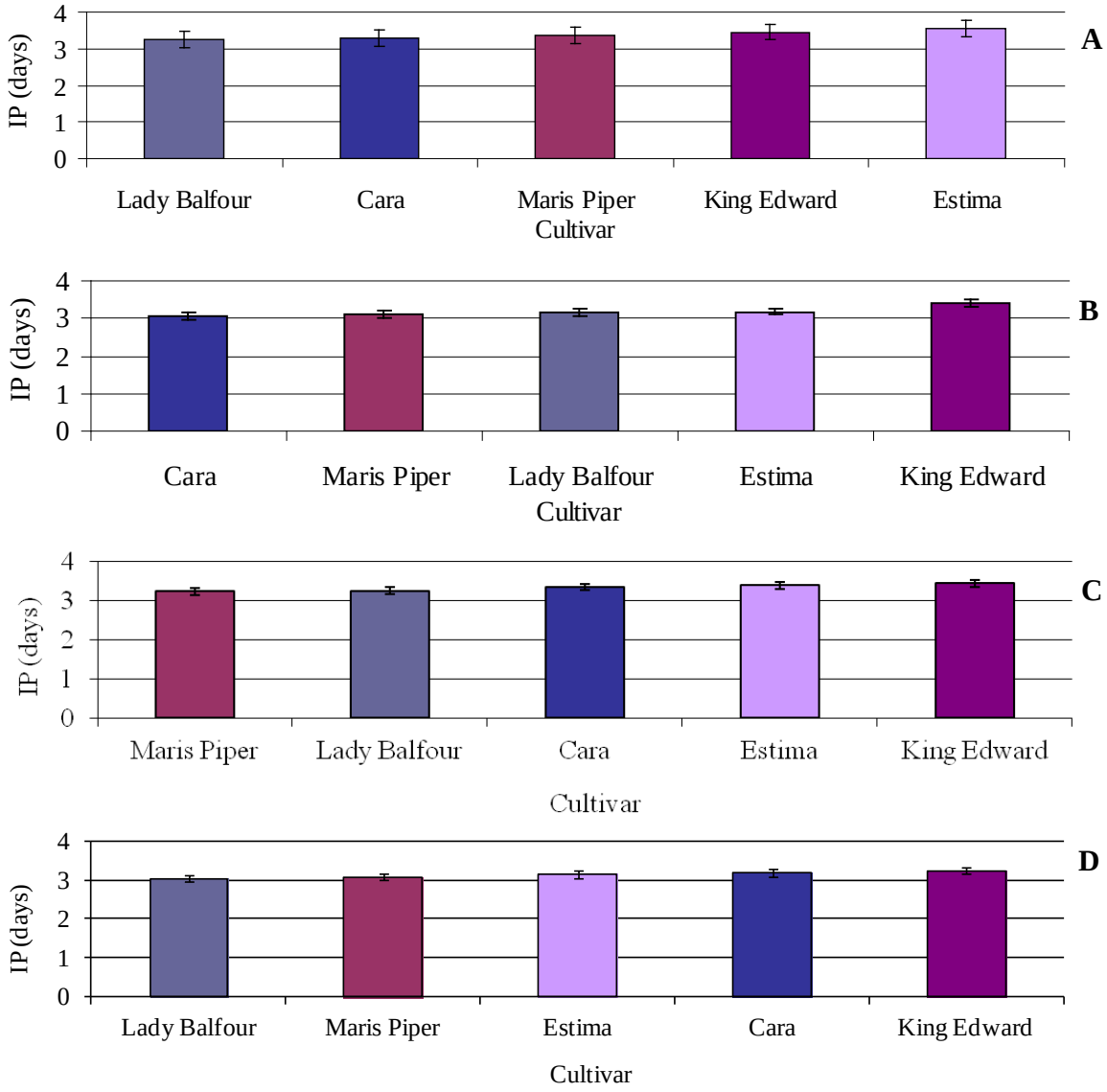


Figure 3.4 – Mean incubation period (IP) for a range of UK *Phytophthora infestans* genotypes on detached potato leaflets (mean of genotype). The error bars represent the stand errors of differences of the means (SE).

A) Test 1 (SE=0.22) B) Test 2 (SE=0.10) C) Test 3 (SE=0.08) D) Test 4 (SE=0.09)

Table 3.3 – Descriptive statistics for aggressiveness tests: testing the variation between the IP of 11 UK *P. infestans* genotypes on five potato cultivars

Test 1 IP - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	16	45.13	2.82	12.87	<.001
Genotype	7	29.72	4.24	17.16	<.001
Cultivar	4	6.32	1.58	0.65	0.635
Genotype x Cultivar	28	4.93	0.17	0.70	0.875
Test 2 IP - Source of Variation	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**Variance Ratio**	**P value**
Isolate	6	35.70	5.95	26.29	<.001
Genotype	4	35.11	8.78	38.25	<.001
Cultivar	4	3.36	0.84	4.18	0.010
Genotype x Cultivar	16	7.45	0.47	2.03	0.015
Test 3 IP - Source of Variation	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**Variance Ratio**	**P value**
Isolate	19	98.1382	5.1652	21.54	<.001
Genotype	8	28.53	3.57	11.28	<.001
Cultivar	4	3.36	0.92	2.29	0.088
Genotype x Cultivar	32	13.58	0.42	1.35	0.099
Test 4 IP - Source of Variation	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**Variance Ratio**	**P value**
Isolate	12	96.66	8.06	57.36	<.001
Genotype	7	5.69	0.81	3.64	<.001
Cultivar	4	2.07	0.52	1.83	0.154
Genotype x Cultivar	28	6.91	0.25	1.10	0.343

3.3.3 Latent period (LP)

3.3.3.1 Isolate

There was a significant difference between the responses of the isolates for LP values in all tests (P<0.001, Figure 3.5, Table 3.4). As before, for the IP values, variation was seen within the genotypes, but not for every test. Genotype 13_A2 showed variation within Tests 1, 3 and 4. In Test 1, genotype 13_A2 was represented by five isolates; 2006_6102A (5.5 days), 2008_6050B (5.5 days), 2008_7038A (5.6 days), 2008_6194A (6.0 days) and 2006_3928A (6.2 days). Both 2008_6102A and 2008_6050B had a significantly shorter LP than 2006_3928A, but not 2006_6194A. 2006_3928A and 2008_6194A were not significantly different from one another. In Test 3, there were four isolates representing genotype 13_A2; the LP of isolate 2006_3928A was significantly the shortest (5.6 days) and 2008_6530C significantly the longest (7.7 days), while 07_39 and 2006_6430A were not significantly different from one another but were significantly different from both 2006_3928A and 2008_6530C. Test 4 had three isolates representing genotype 13_A2; the LP of isolates 2008_6082F (5.1 days) and 2006_3928A (5.1 days) were significantly shorter than that of 2008_6250A (7.1 days). No variation was seen in Test 2 because only on isolate represented genotype 13_A2. Genotype 6_A1 only showed variation within the genotype in Tests 3 and 4. In Test 3, 2008_6090A (5.2 days), 2008_6610E (7.1 days) and 2006_4100A (7.8 days) all had significantly different LP values (Figure 3.4.C). In Test 4, the two isolates representing genotype 6_A1 differed by a large amount; 2008_6090A had an LP value of 5.5 days and 2008_6498A had a LP value of 7.5 days. Other genotypes showed this tendency to have variation in some tests and not in others, i.e. genotype 2_A1 only showed variation within its isolates in Test 3 but not in Test 1 (Test 2 and 4 only contained one isolate of genotype 2_A1).

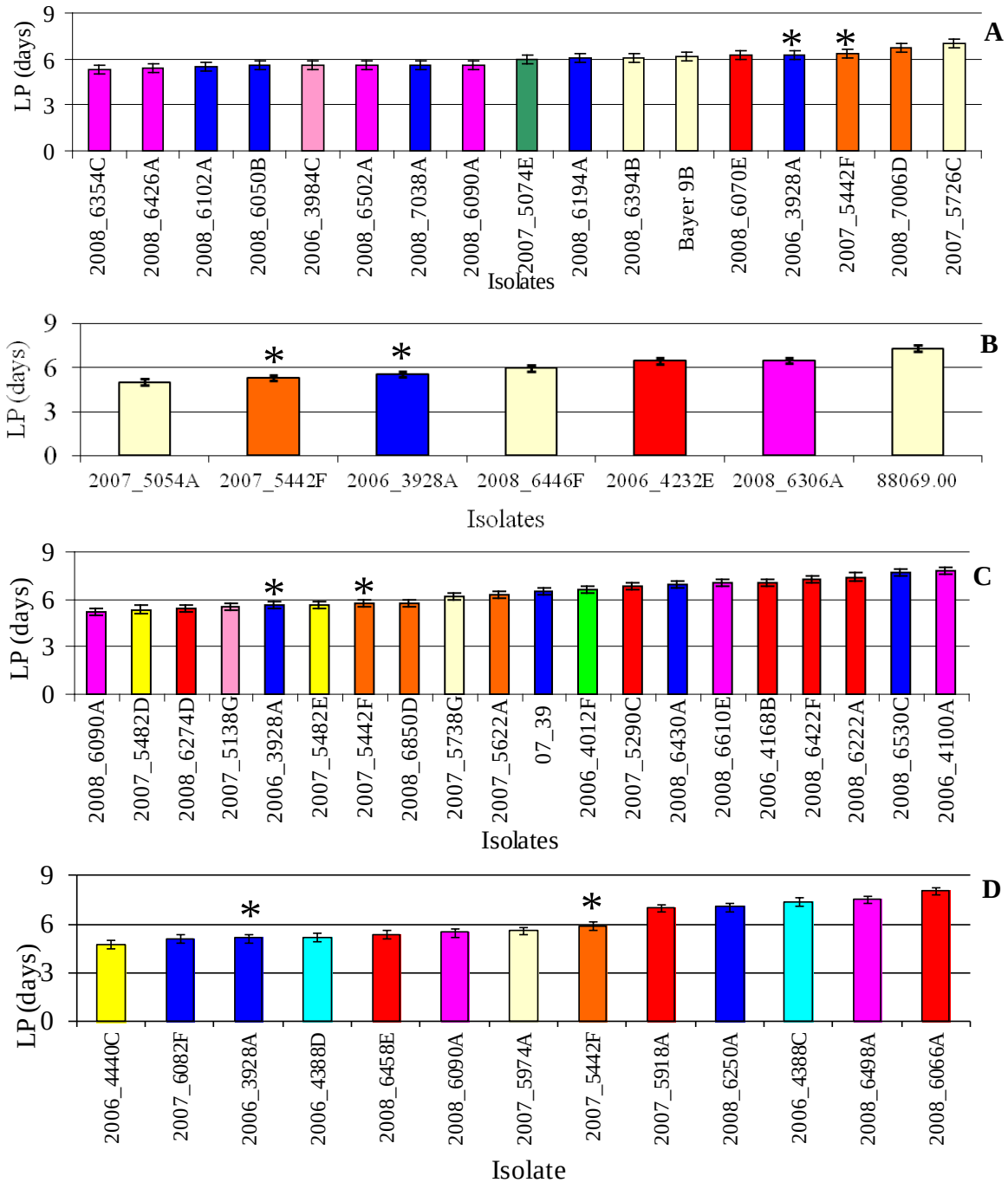


Figure 3.5 – Mean latent period (LP) values for range of UK *Phytophthora infestans* isolates on detached potato leaflets (mean of cultivar). The error represents the standard errors of the difference of the means (SE)

A) Test 1 (SE=0.29) B) Test 2 (SE=0.20) C) Test 3 (SE=0.23) D) Test 4 (SE=0.25)

*= Reference isolates

1_A1 2_A1 3_A2 6_A1 7_A1 8_A1 10_A2 13_A2 17_A2 A1Misc A2 Misc

3.3.3.2 Genotype

There was a significant difference between the responses of genotypes for LP values in all tests (P<0.001 Figure 3.6, Table 3.4). Genotype 13_A2 did not have the significantly shortest LP in Tests 1, 3 and 4. In Test 2, genotype 13_A2 did have one of the shortest LP values out of the genotypes present in that test. Genotype 6_A1 did sporulate the quickest in Test 1 as it had the shortest LP value, but in Tests 2, 3 and 4 the LP value was of an intermediate to high value. Although the ranges of the genotypes 13_A2 and 6_A1 were similar, in one out of four tests genotype 6_A1 had a significantly shorter LP. No genotype had the consistently shortest LP in all tests. Genotype 10_A2 had the shortest LP value in Tests 3 and 4 but the difference between the two LP values of genotype 10_A2 is large; 5.5 days and 4.8 days for Tests 3 and 4 respectively. In Test 4, genotype 10_A2 had a significantly shorter LP than all other genotypes in that test, whereas in Test 3, the LP value for genotype 10_A2 was not significantly different from 1_A1 and 2_A1 but significantly different from the other genotype present in the test. In Tests 1, 2 and 3, genotype 8_A1 had consistently the longest or a longer LP values than the other genotypes within the tests. Test 4 was the exception as it had the second shortest LP value. Genotype 7_A1 had one of the longest LP values in Tests 3 and 4.

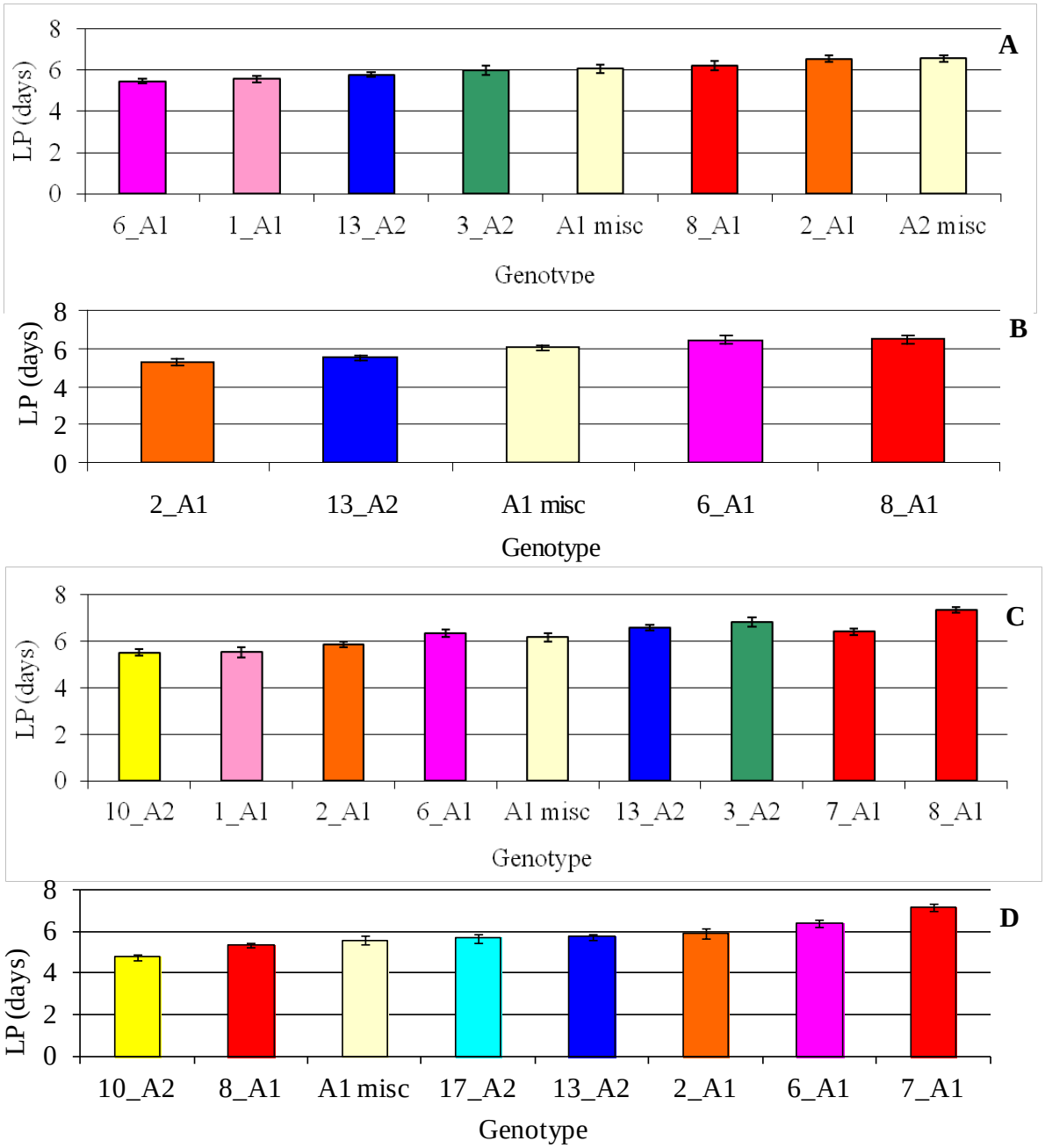


Figure 3.6 – Mean latent period (LP) values for a range of UK *Phytophthora infestans* genotypes on detached potato leaflets (mean of cultivar). The error bars represent the standard error of differences of the means (SE). Number of isolates representing each genotype in shown after the SE value.

A) Test 1 SE=6_A1 0.11 (n=4),1_A1 0.17 (n=1), 13_A2 0.10 (n=5), 3_A2 0.24 (n=1), A1 Misc 0.21 (n=1), 8_A1 0.22 (n=1), 2_A1 0.16 (n=2) and A2 Misc 0.17 (n=2)

B) Test 2 SE=2_A1 0.19 (n=1), 13_A2 0.13 (n=1), A1 Misc 0.14 (n=3), 6_A1 0.19 (n=1) and 8_A1 0.24 (n=1)

C) Test 3 SE=10_A2 0.14 (n=2), 1_A1 0.22 (n=1), 2_A1 0.11 (n=3), 6_A1 0.17 (n=3), A1 Misc 0.19 (n=1), 13 A2 0.12 (n=4), 3_A2 0.20 (n=1), 7_A1 0.14 (n=3) and 8_A1 0.12 (n=2)

D) Test 4 SE=10_A2 0.12 (n=1), 8_A1 0.11 (n-1), A1 Misc 0.23 (n=1), 17_A2 0.21 (n=2), 13_A2 0.15 (n=3), 2_A1 0.25 (n=1), 6_A1 0.19 (n=2) and 7_A1 0.20 (n=2)

3.3.3.3 Cultivar

LP was significantly affected by cultivar in all tests ($P<0.001$, Figure 3.7, Table 3.4) but there was no significant interaction between genotype and cultivar (Table 3.4). On average, the order of resistant to susceptible cultivars for LP was Lady Balfour (6.4 days), Cara (6.2 days), Estima (6.0 days), Maris Piper (5.7 days) and King Edward (5.7 days). Maris Piper and King Edward were consistently the most susceptible: genotypes infecting the two cultivars had short LP values. Lady Balfour had an intermediate resistance in Tests 2, 3 and 4 but in Test 1 it was the most resistant cultivar as most genotypes took longer to sporulate on it when compared to the other cultivars. Cara was the most resistant cultivar in Tests 2 and 3, whereas in Tests 1 and 4 it was the second most resistant. Resistance of Estima varied from intermediate in Test 1, second most resistant in Tests 2 and 3 and the most resistant in Test 4.

Interactions between isolate and cultivar were seen for Test 2 and 3 but not for Test 1 and 4 (data shown in Appendix ii). Isolates infecting Maris Piper generally had longer LP values compared to when the same isolates were infecting Cara. This was not true for all isolates, for example, isolate 2006_3928A had an LP value of 6.0 and 5.8 when infecting Maris Piper and Cara respectively.

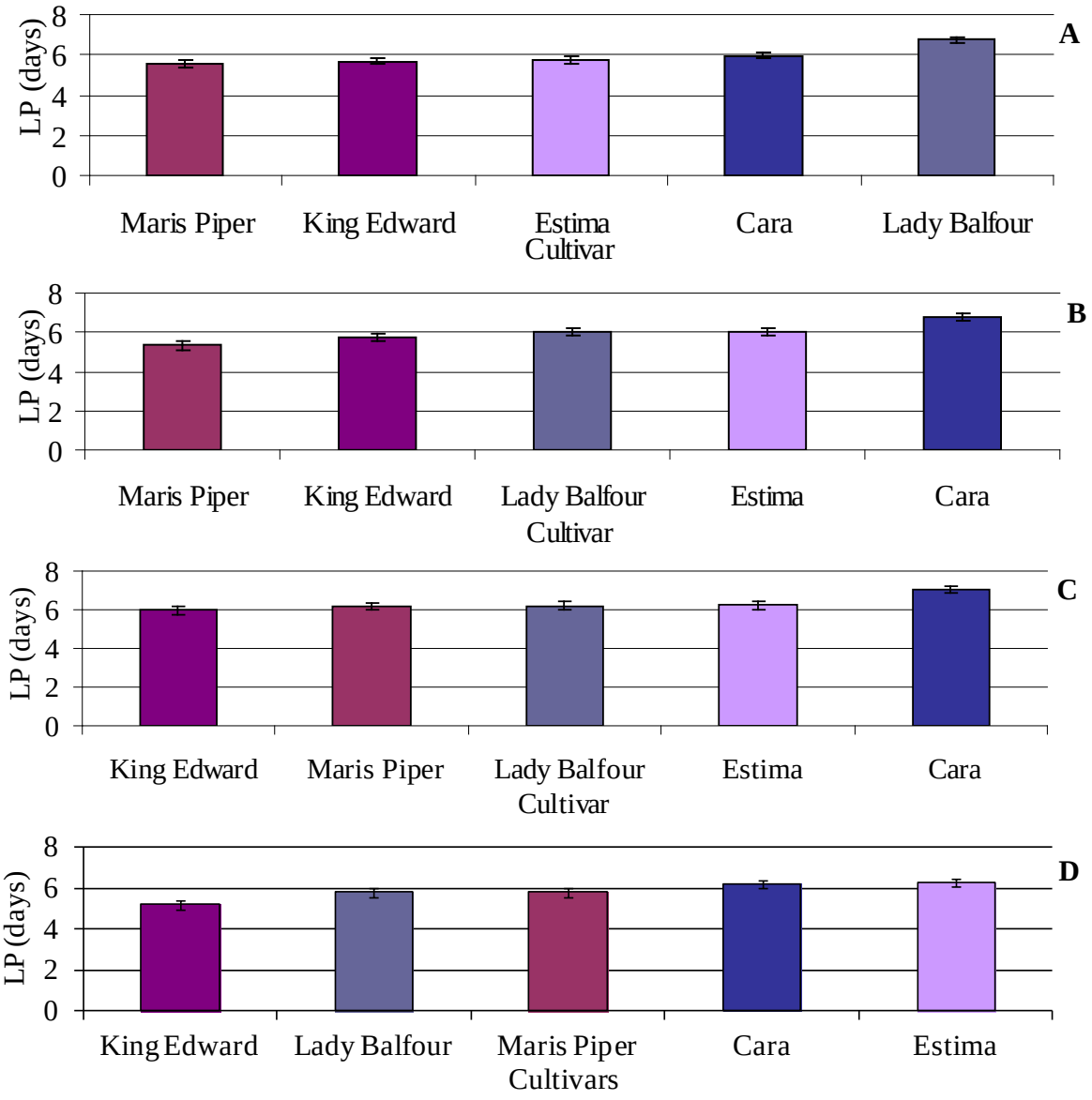


Figure 3.7 – Mean LP for a range of UK *Phytophthora infestans* genotypes on detach potato leaflets (mean of genotype). The error bars represent the standard error of the difference of the means (SE)

A) Test 1 (SE=0.16) B) Test 2 (SE=0.21) C) Test 3 (SE=0.20) D) Test 4 (SE=0.21)

Table 3.4 – Descriptive statistics for aggressiveness tests: testing the variation between the LP of 11 UK *P. infestans* genotypes on five potato cultivars

Test 1 LP - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	16	115.69	7.23	5.81	<.001
Genotype	7	83.00	11.85	9.23	<.001
Cultivar	4	90.48	22.62	17.72	<.001
Genotype x Cultivar	28	29.908	1.068	0.82	0.729
Test 2 LP - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	6	114.90	19.15	33.23	<.001
Genotype	4	34.52	8.63	6.96	<.001
Cultivar	4	45.55	11.39	12.59	<.001
Genotype x Cultivar	16	26.09	1.63	1.35	0.174
Test 3 LP - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	19	395.69	20.82	25.74	<.001
Genotype	8	160.70	20.08	16.46	<.001
Cultivar	4	80.70	20.17	16.53	<.001
Genotype x Cultivar	32	42.95	1.34	1.14	0.280
Test 4 LP - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	12	441.48	36.79	39.89	<.001
Genotype	7	164.05	23.44	16.27	<.001
Cultivar	4	57.76	14.44	8.12	<.001
Genotype x Cultivar	28	22.19	0.79	0.52	0.979

3.3.4 Area under the lesion expansion curve (AULEC)

3.3.4.1 Isolate

There was a significant difference between the responses of the isolates for AULEC values in all tests ($P<.001$, Figure 3.8, Table 3.5). Variation was seen for genotypes 13_A2 and 6_A1 in Tests 1, 3 and 4. In Test 1, five isolates represented genotype 13_A2; 2008_6102A (13_A2_2) was significantly different from 2008_6194A (13_A2_1). Isolate 2008_6050B (13_A2_2), 2006_3928A (13_A2_1) and 2008_7038A (13_A2_1) were not significantly different from one another but only 2008_7038A had a significantly smaller AULEC than 2008_6102A. In Test 3, genotype 13_A2 was represented by four isolates; isolate 2006_3928A had significantly the largest AULEC value, isolate 2008_6530C had significantly the smallest AULEC value and both 2008_6430A and 07_39 had intermediate AULEC values which were not significantly different from one another but were significantly different from isolates 2006_3928A and 2008_6530C. In Test 4, three isolates represented genotype 13_A2; isolates 2006_3928A and 2008_6082F had significantly larger AULEC values than 2008_6250D. Both 2006_3928A and 2008_6250D were a part of the 13_A2_1 subset and were significantly different from one another. 2008_6082F was the only genotype 13_A2_5 isolate in this study. In Test 1, genotype 6_A1 had four representative isolates; isolate 2008_6090A had a significantly larger AULEC value than the other three isolates. In Test 3, two isolates represented genotype 6_A1; isolate 2008_6090A and 2008_6610E had significantly different values. In Test 4, genotype 6_A1 had two isolates representing it; 2008_6090A and 2008_6498A were significantly different from one another. Other genotypes showed significant variation within the genotypes, for example A2 Misc in Test 1, A1 Misc in Test 2, genotype 7_A1 and 8_A1 in Test 3 and genotype 17_A2 in Test 4. Conversely, some genotypes did not show any variation within the genotype, for example, genotype 10_A2 in Test 3. No

clustering of any one particular genotype was seen in the tests except in Test 1. In Test 1, isolates that represented genotype 13_A2 were clustered together all showing intermediate to low AULEC values, although there were significant differences between the isolates. Additionally in Test 1, isolates of the genotype 6_A1 had some of the largest AULEC values within this test and were clustered together although, again, significant differences were seen between the isolates. No isolate had consistently the largest AULEC value.

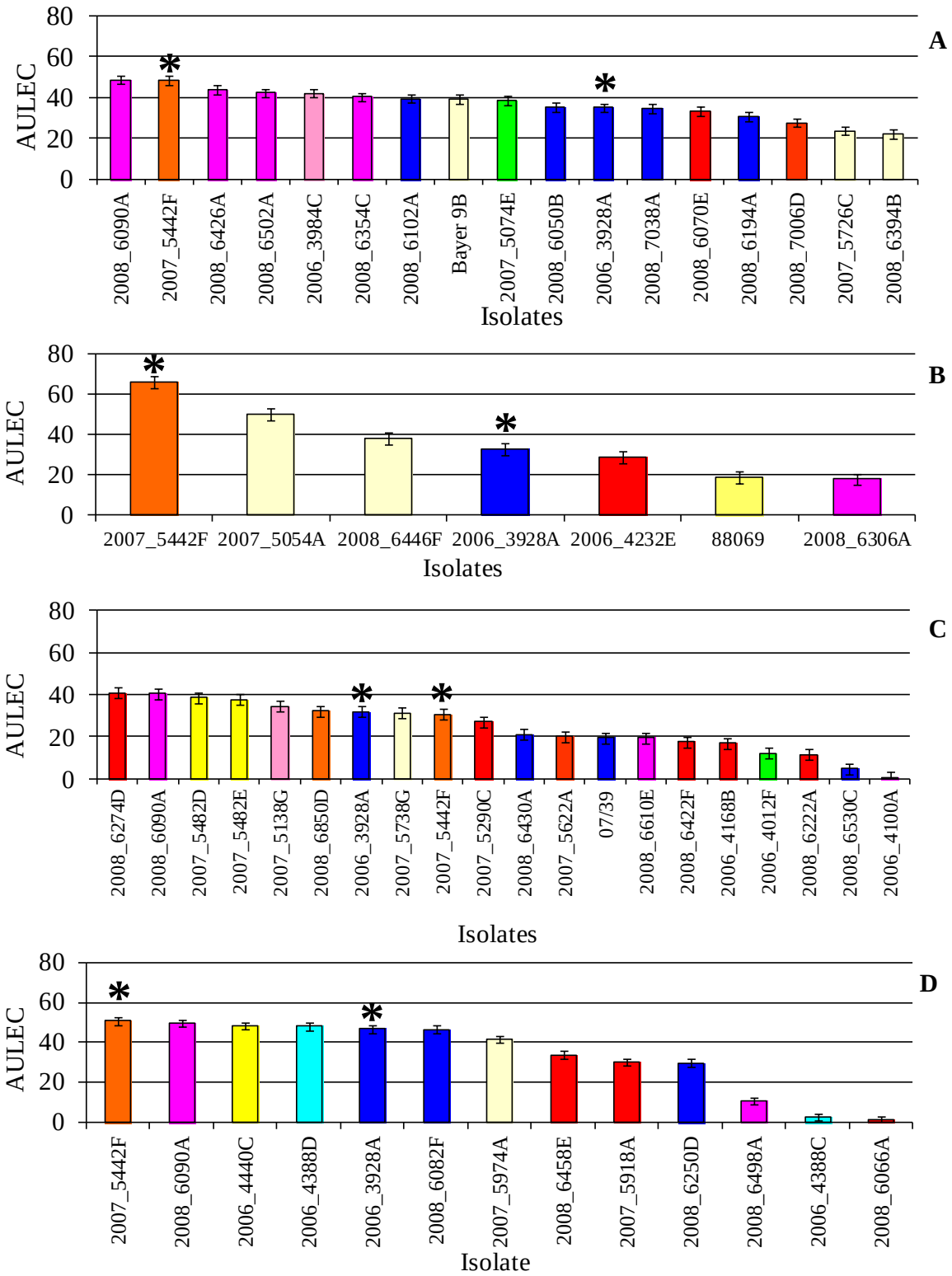


Figure 3.8 – Mean AULEC values 7 days post inoculation of a range of UK *Phytophthora infestans* isolates on detached potato leaflets (mean of cultivar). The error bars represent the standard errors of differences of the means (SE)

*= Reference isolates

A) Test 1 (SE=2.18) B) Test 2 (SE=2.96) C) Test 3 (SE=2.58) D) Test 4 (SE=0.86)

1_A1 2_A1 3_A2 6_A1 7_A1 8_A1 10_A2 13_A2 17_A2

A1Misc A2 Misc

3.3.4.2 Genotype

There was a significant difference between the responses of the genotypes for AULEC values in all tests (P<0.001, Figure 3.9, Table 3.5). Genotype 13_A2 had an intermediate AULEC value in all tests, although the ranking was not consistent between the tests and neither were the AULEC values. Genotype 6_A1 was not consistent in the ranking in the tests, for example, in Test 1 genotype 6_A1 had the largest AULEC value which was significantly larger than the AULEC of genotype 13_A2. In Test 2, genotype 6_A1 had significantly the smallest AULEC value compared to all other genotypes in that test and in Test 3 and 4 the AULEC was of an intermediate value. There were other examples of genotypes not being consistent through the tests, i.e. genotype 3_A2 had the smallest AULEC in Test 3 but in Test 1 it had one of the largest AULEC values. Genotype 8_A1 had intermediate AULEC values in all tests. Genotype 7_A1 had an intermediate AULEC in Test 3 but the smallest AULEC value in Test 4.

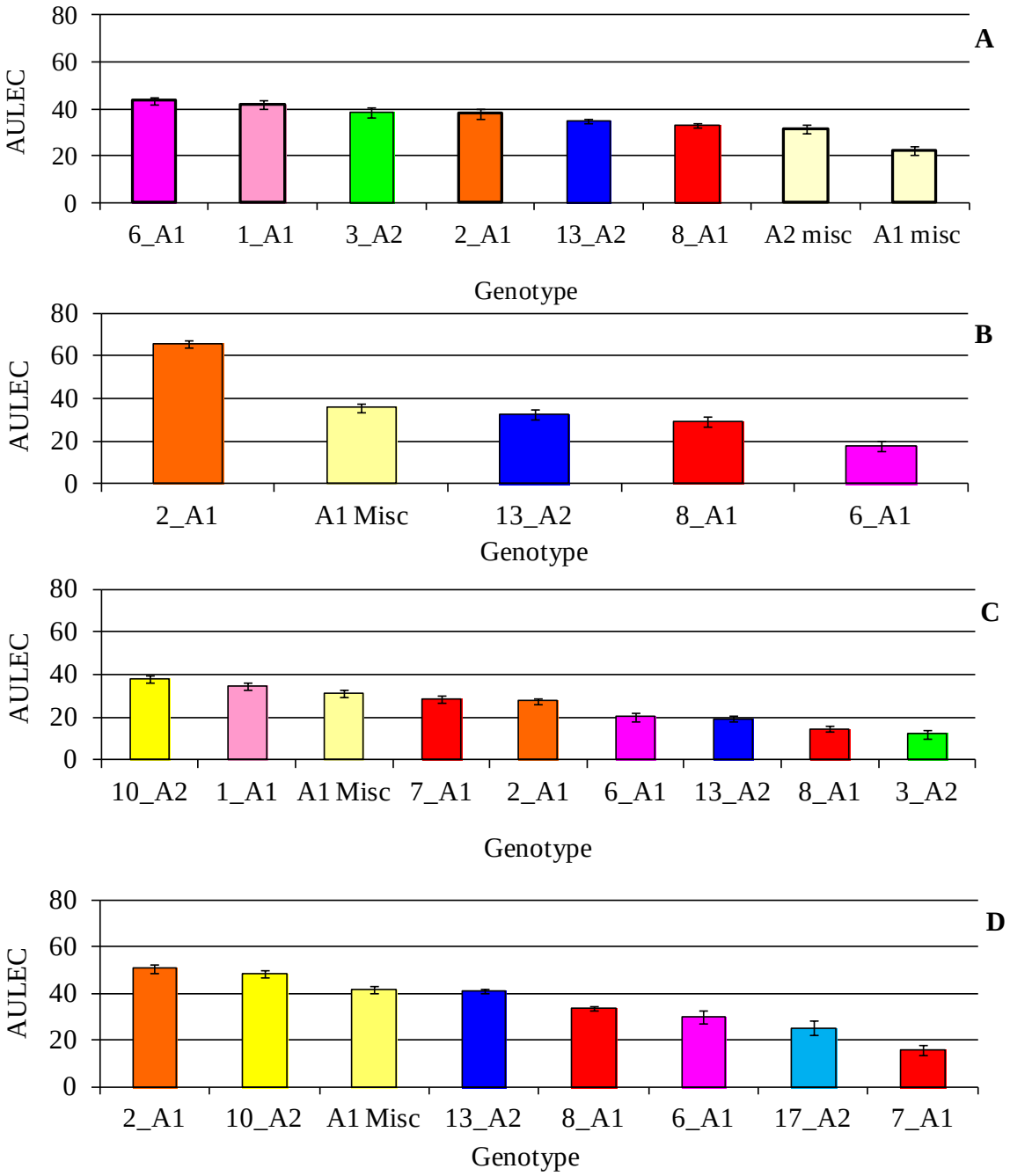


Figure 3.9 – Mean AULEC values 7 days post inoculation of a range of UK *Phytophthora infestans* genotypes on detached potato leaflets (mean of cultivar). The error bars represent the standard errors of differences of the means (SE). Number of isolates representing each genotype is stated after the SE.

A) Test 1 SE=6_A1 1.50 (n=4), 1_A1 1.71 (n=1), 3_A2 2.10 (n=1), 2_A1 2.16 (n=2), 13_A2 0.89 (n=5), 8_A1 1.05 (n=1), A2 Misc 1.79 (n=1) and A1 Misc 1.86 (n=2)

B) Test 2 SE=2_A1 1.60 (n=1), A1 Misc 2.07 (n=3), 13_A2 2.50 (n=1), 8_A1 2.33 (n=1) and 6_A1 2.47 (n=1)

C) Test 3 SE=10_A2 1.71 (n=2), 1_A1 1.79 (n=1), A1 Misc 1.59 (n=1), 7_A1 1.69 (n=3), 2_A1 1.53 (n=3), 6_A1 1.94 (n=3), 13_A2 1.22 (n=4), 8_A1 1.33 (n=2) and 3_A2 1.96 (n=1)

D) Test 4 SE=2_A1 1.70 (n=1), 10_A2 1.53 (n=1), A1 Misc 1.43 (n=1), 13_A2 1.18 (n=3), 8_A1 1.00 (n=1), 6_A1 2.85 (n=2), 17_A2 3.16 (n=2) and 7_A1 2.14 (n=2)

3.3.4.3 Cultivar

Cultivar had no significant effect on the AULEC values for Test 1 (P=0.08, Figure 3.10, Table 3.5). For Test 2, 3 and 4, significant differences were seen between the cultivars (P<.001, P=0.01 and P<.001 respectively, Figure 3.10, Table 3.5); Cara was the most resistant cultivar with the smallest AULEC values, in most cases the AULEC was significantly smaller than those of Maris Piper, King Edward and Lady Balfour; which were the most susceptible cultivars throughout the tests. Estima had intermediate levels of resistance in Tests 2, 3 and 4. There were no significant interactions between genotype and cultivar for Tests 1, 3 and 4 whereas there was for Test 2 (Table 3.3), this was the same for interactions between isolate and cultivar also (see Appendix ii). In Test 2, when comparing the AULEC value of genotype 13_A2 on the five cultivars there was no difference between, the AULEC range from 27.67 for Maris Piper and 37.85 for Lady Balfour showing that genotype 13_A2 produces a similar amount of disease on all cultivars. Genotype 6_A1 had significantly more disease on Lady Balfour than Estima. This was not the case for genotype A1 Misc and genotype 8_A1 where Maris Piper and King Edward normally had the most disease. Genotype 2_A1 showed no significant difference in growth on different cultivars, like genotype 13_A2, but the amount of disease present was much greater than genotype 13_A2.

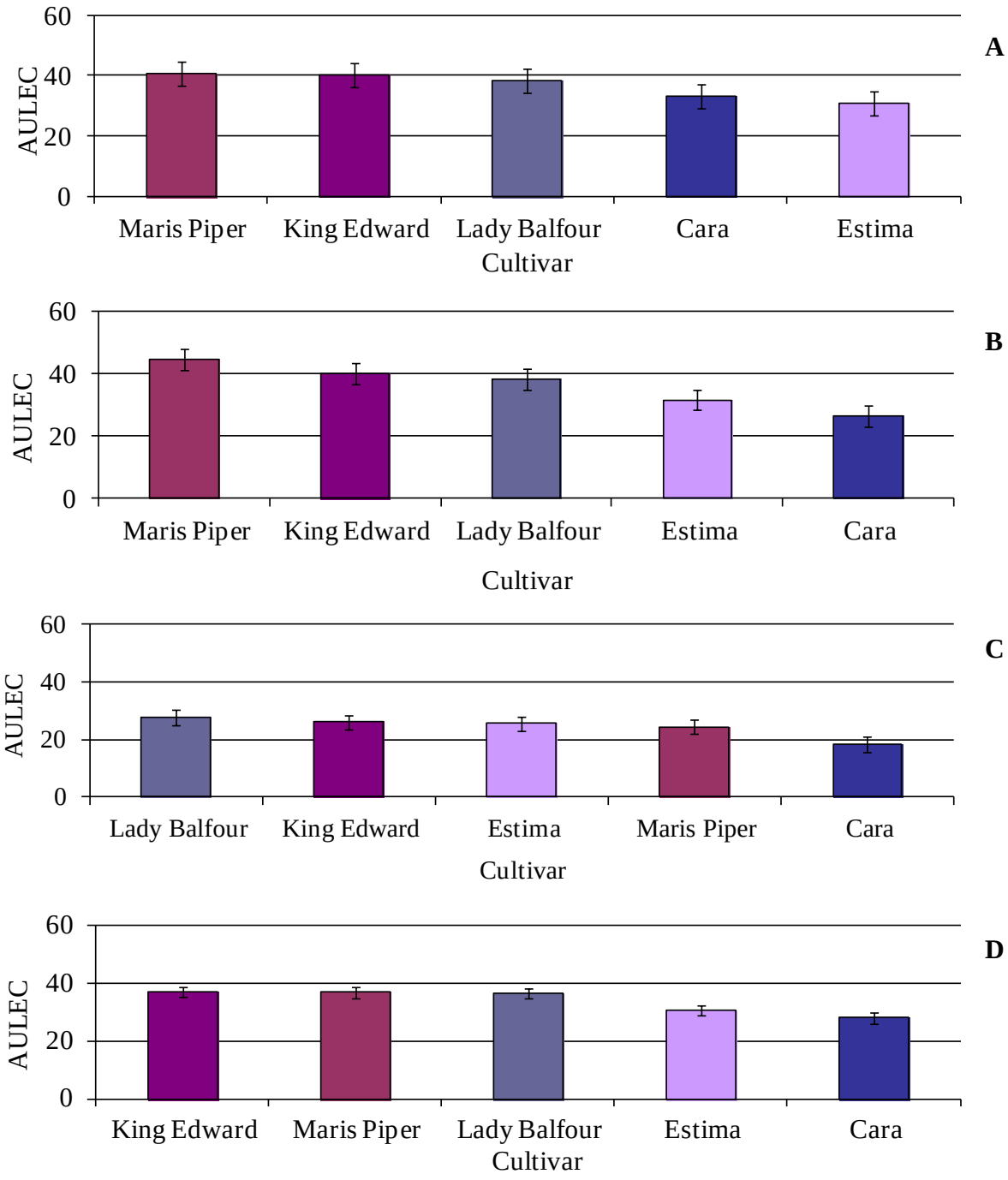


Figure 3.10 – Mean AULEC values 7 days post inoculation of a range of UK *P. infestans* genotypes on detached leaflets of five cultivars (mean of genotype). The error bars represent the standard errors of the differences of the means (SE)

A) Test1 (SE=3.954) B) Test 2 (SE=3.318) C) Test 3 (SE=2.56) D) Test 4 (SE=1.793)

Table 3.5 – Descriptive statistics for aggressiveness tests: testing the variation between the AULEC of 11 UK *P. infestans* genotypes on five potato cultivars

Test 1 AULEC - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	29	28065.82	1754.11	24.60	<.001
Genotype	7	15660.64	2237.23	23.56	<.001
Cultivar	4	7537.14	1884.28	2.36	0.08
Genotype x Cultivar	28	3223.83	115.14	1.21	0.212
Test 2 AULEC - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	5	52958.80	8826.50	67.15	<.001
Genotype	4	8129.55	2032.38	45.02	<.001
Cultivar	4	2409.78	602.44	13.34	<.001
Genotype x Cultivar	16	6.65.70	379.10	1.93	0.021
Test 3 AULEC - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	19	79368.29	4177.28	41.81	<.001
Genotype	8	32792.4	4099.1	22.36	<.001
Cultivar	4	6119.7	1529.9	3.9	0.014
Genotype x Cultivar	32	4453.80	139.20	0.76	0.829
Test 4 AULEC - Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P value
Isolate	12	117977.2	9831.43	189.36	<.001
Genotype	7	46194	6599.1	24.35	<.001
Cultivar	4	5510.7	1377.7	10.98	<.001
Genotype x Cultivar	28	1755.20	62.70	0.23	1.000

3.4 Discussion

3.4.1 Aggressiveness tests

The difficulty in standardising the aggressiveness tests was a major problem. Lehtinen *et al*. (2009) also encountered this problem. In 2003, *P. infestans* isolates were collected from the potato growing areas of Denmark, Finland, Norway and Sweden with the total number of isolates collected being 61, 264, 329 and 89, respectively. Laboratories in Norway, Sweden and Finland conducted aggressiveness tests on potato cultivars Bintje and Matilda for 25 isolates that were randomly picked from each sample site and an extra 5 Danish isolates, which were tested in all laboratories. Tests in different laboratories were carried out at different times of the year and different methods of producing sporangia for inoculum were used. In Sweden, tubers were used to grow sporangia which were then rinsed off with distilled water, whereas in Finland and Norway sporangia from agar plates were used to create the sporangial suspensions, but the same concentration of sporangia was used to inoculate leaflets in all laboratories. All potato plants were grown 2 litre plant pots in a glasshouse set at 15°C with day length of 16 hours which was a created with a mixture of natural and artificial light. Expanded leaflets from the upper layers of the canopy of plants aged from 6–7 weeks were used in all laboratories. The experimental design used was a resolvable row-column design which involved eight columns (eight different test plants) each with five leaflets from each cultivar; in Norway and Sweden this was done in growth chambers and in Finland it was conducted on a glasshouse bench. The aggressiveness criteria that were measured were infection frequency, latent period, lesion growth rate and sporulation capacity. All methods were standardised in all laboratories except for details such as what the leaflets were placed in and how sporangia were transferred for counting. Inconsistency between laboratories was seen even when the methods were standardised; in Norway the infection efficiency of the five Danish isolates was 2.7%

compared to 1% in the Swedish and Finnish laboratories. For LP on the cultivar Matilda the five Danish isolates showed significant differences between tests done in Norway and Finland, while differences in the sporulation capacity could not be seen due to the inconsistencies between the cultivars. Variation was thought to be due to laboratory effects and experimental error (Lehtinen *et al*., 2009).

For the present study, the standard isolates in each test gave significantly different results in terms of lesion expansion so the study could not be analysed as a whole and instead had to be treated as four separate tests. Day and Shattock (1997) looked at the fitness index (infection frequency x mean number of sporangia per lesion) of three standard isolates in order to be able to compare three separate experiments. In Day and Shattock's (1997) study variation between experiments for the standard isolates was seen but the ranking of the isolates was consistent between the standard isolates used, but was not the case in this aggressiveness study. Day and Shattock (1997) transformed the data to conform to the standard isolates. For this study, differences between tests were seen with no consistent trends between the isolates i.e. isolate 2006_3928A showed no significant difference between Tests 1, 2 and 3, but isolate 2007_5442F were significant different for Tests 1, 2 and 3. Although environmental factors were kept as constant as possible even small variations may have had a large influence on the parameters that were measured, making it hard to get reproducibility. Many factors could have affected this study. One of the most obvious is that each test was conducted at a different time of the year, Test 1 was conducted in late June, Test 2 in late July, Test 3 in late August and finally Test 4 in mid October. For each experiment, leaflets from different plants were used, all adding potential variation to the experiments. Although the utmost was done to keep the detached leaflets used as uniform as possible, e.g. from plants of the same age, from the same position in the plant and of the same size, it is impossible to estimate the amount of variation this caused within the

experiments. In addition, the time of year would have affected the growth of the plant with differing quality and quantity of light and day length occurring at the different months of the year. Lehtinen *et al*. (2009) suggested a lot of the differences within the columns of the experiments were due to inoculum preparation and production. For each test, inoculum was always produced using the same method but it was made from different sporulating lesions and with an increased number of passages as the tests went on. The age of the lesion and the sporangia on those lesions would not necessarily be of the same age each time, and age of the sporangia affects zoospore release.

Standardising assays has proved to be difficult even for virulence tests. Virulence tests require a score of the presence or absence of a lesion as they test whether an isolate can overcome a particular R gene within a cultivar that contains only one R gene. Andrivon *et al*. (2011) found that when assessing the virulence of 10 European *P. infestans* isolates across 12 laboratories in Europe using a standardised protocol and a common set of host differentials, some comparisons could be made, but inconsistencies were found. Identification of virulence to R1, R3, R4, R10 and R11 was mostly consistent in all laboratories, but the other R genes proved to have more discrepancies between the laboratories, particularly R6 and R9.

Due to the separation of the isolates into different tests it was much harder to distinguish the real variation between the isolates and the genotypes. It proved impossible to assess the overall aggressiveness of genotypes because the rankings were not consistent between tests, so only comments on trends could be made rather than comments on statistical evidence. The rankings of the genotypes within each test were based on the isolates present and this does not allow true representation of the genotypes. For example, in Test 2 most genotypes were represented by one isolate alone. In addition, one test may have contained the most aggressive isolates of a particular genotype and another test the least aggressive. Genotype 7_A1 is an

example of this, in Test 3 genotype 7_A1 had a high to intermediate level of aggressiveness but in Test 4 the level of aggressiveness was low. If all isolates were tested together a more thorough assessment of aggressiveness would have been carried out. To do this, one person should be designated to prepare the inoculum in order to remove the variation of having different people and methods used. All isolates would be passaged the same number of times and all lesions would be of the same age. To test a collection of isolates all together would have been more time-consuming, space-consuming and require more man power compared to four smaller separate tests. A balanced incomplete block design could have also been used, as seen in Carlisle *et al*. (2002). This design is used for experiments when there is a large sample number that needs to be tested but not all can be tested in each experiment and accounts for the variation within the isolates as pairs of treatments occur together a number of times. However, it is not clear from the results obtained in this study to what extent aggressiveness can be considered as a characteristic of a genotype and how many isolates of a particular genotype would need to be tested to give a truly representative result.

3.4.2 Cultivar

The levels of foliar cultivar resistance demonstrated in the study and the cultivars' published resistance ratings matched in most cases. As stated in Chapter 3.1.3, the resistance ratings for foliar blight for Cara, Maris Piper, Estima, Lady Balfour and King Edward are now 5, 4, 4, 4 and 3 respectively. In most cases, the mean AULEC values for Cara showed that less disease occurred on this cultivar and thus this is comparable to the resistance ratings. Maris Piper, Lady Balfour and King Edward had the most disease present in tests and were no different from each other. Estima generally had higher AULEC values than Cara, except in Test 3, but values did not differ significantly. In Test 3, Estima had significantly higher levels of disease than Cara.

There was no significant effect of cultivar on IP (except in Test 2 where IP on King Edward was significantly longer than the other four cultivars) so this parameter is not useful as a measure of cultivar resistance since statistically all cultivars acted the same and the ranking order of the data was very different from that of the resistance ratings. This could be because, when *P. infestans* infects a potato plant, a cascade of plant defences occurs, thus slowing the subsequent growth of isolates that are unable to overcome them easily, so although an isolate may infect the plant quickly, this has no bearing on how well it will then grow within the plant tissue. Cultivar influenced the LP of genotypes and the only difference between the order of the resistance ratings and the data in this study was that in Test 1 Lady Balfour had the significantly longest LP; *ergo* it would be classed as the most resistant of the cultivars in regard to LP instead of, as the resistance ratings predict, Cara.

In the study by Cooke *et al.* (2012a) the smallest lesions were produced on Cara, whereas Lady Balfour was the most resistant. Mostly, the largest lesions for both the 2007 and 2010 aggressiveness tests were produced on King Edward. In this study, *P. infestans* genotypes that infected more resistant cultivars, i.e. Cara and Lady Balfour, generally had lower AULEC values and longer LPs compared to those infecting more susceptible cultivars i.e. King Edward and Maris Piper. More data could have been generated if the study had been carried out on larger leaflets.

The lack of interaction seen between genotype and cultivar in Tests 1, 3 and 4 could be due to the large number of isolates tested which may mask subtle differences due to the wide variation seen amongst isolates of a genotype. Interactions between isolate and cultivar were detected more often due to the values not being grouped into genotypes for analysis. In Test 2, fewer isolates were tested which may mean the differences could be detected more easily. There was no significant difference in both the IP value and AULEC for genotype 13_A2 between the cultivars which contradicts the idea that

genotype 13_A2 does overcome previously resistant plants e.g. cultivar Stirling. Cultivar Stirling, although not widely grown, does contain a major R gene that was overcome by genotype 13_A2, and not other genotypes.

3.4.3 UK populations

3.4.3.1 Genotypes 13_A2

Genotype 13_A2 has been the dominant genotype in the UK populations from 2007–2010, but the data in this study cast doubt on whether this is solely because genotype 13_A2 is more aggressive than other genotypes. At 15°C, genotype 13_A2 was not the most aggressive compared to other genotypes as the AULEC values showed that it caused an intermediate amount of disease. When comparing the data from this study conducted at 15°C with those from the study carried out in 2007 (Cooke *et al.*, 2012a) at 13°C and 18°C, the results at 15°C are intermediate between the 13°C and the 18°C data. At 13°C, genotype 13_A2 was more aggressive compared to the other genotypes whereas at 18°C the differences between the genotypes were small as all genotypes grew well at the higher temperature. This is in agreement with the idea that 13_A2 is more aggressive at lower temperatures, which may have contributed to its dominance of the GB population. However the detached leaflet studies showed that it was not more aggressive on potato foliage at 15°C and above. It is hard to compare genotype 13_A2 to all genotypes in this study because of the variation seen between the tests and not all genotypes were present in every test. Genotype 13_A2 was not the most aggressive genotype at 15°C so this does not explain why it would have displaced the genotypes in the previous population. Several genotypes had significantly larger AULEC values at 15°C than genotype 13_A2 such as genotypes 1_A1, 2_A1, 10_A2 and A1 Misc. Cooke *et al*. (2010) stated that a 10%-15% increase in lesion size and a 12 hour shorter LP could give a genotype an advantage in the field. At 13°C, genotype 13_A2 did have

lower LP values and larger lesion sizes than other genotypes, suggesting that genotype 13_A2 would have an advantage earlier on in the season when the temperatures are cooler, being able to colonise and sporulate sooner than other genotypes allowing genotype 13_A2 to dominate.

The A2 mating type was found in Europe in the 1980s, was first reported in England and Scotland in 1985 (Shaw *et al*., 1985; Malcolmson, 1985), and has been present in the UK population for the past 27 years. This could have potentially led to the formation of oospores that gave rise to genotype 13_A2 or it could have resulted from sexual recombination on mainland Europe and then been introduced into the UK (Cooke *et al.*, 2012a). The combination of the adaptation to grow at the lower temperatures, the resistance to the fungicide metalaxyl and, with the large amount of repetitive DNA, higher copy number of genes, slight changes in amino acid sequence in proteins and changes in expression patterns, which have been shown to be present in the *P. infestans* genome (Haas *et al*., 2009), gave genotype 13_A2 the opportunity to dominate the UK population (Cooke *et al*., 2012a). The level of fitness it has over the other genotypes could have potentially been greater due to a lack of deleterious mutations lost through sexual recombination, although most sexual progeny will not be more fit than their parents. A variety of offspring would be produced but selection pressures will dictate which go on to be prolific in the population. The potential level of mutation in a potato field would be high considering how many sporangia are produced; this level of fecundity is essential as not all sporangia would go on and infect a host. In order for *P. infestans* to succeed and remain a threat to the potato it constantly needs to adapt and evolve as specialism could lead to a reduction in fitness; this is known as the Red Queen hypothesis (Clay and Kover, 1996).

3.4.3.2 Variation

The components of foliar aggressiveness will affect the genotypic structure of the *P. infestans* population. Isolates that are more aggressive will colonise the crop faster, producing more sporangia and in turn, out-compete the other isolates. Aggressiveness is not always associated with fitness as an isolate that is highly aggressive on tubers may not survive over winter (as discussed in Chapter 1.9). The shifts in the populations each year show which genotypes are out-competing the rest. Each genotype will have unique differences in the genome and these may be related to differences the components of aggressiveness, but as this study has shown there is also substantial variation between isolates of the same genotype. Within isolate variation has been studied by Caten and Jinks, (1968). In their study, single zoospores, single sporangia and single hyphal tips from three isolates were examined for differences in growth, morphology and sporangial production; only one isolate was discussed in detail in the paper. It was found that there was variation in morphology between single zoospore progeny from this isolate. Growth on rye meal plates varied from just a few millimetres to total colonisation of the plate: the growth rates were shown to differ significantly. When growing from a single sporangium or from a single hyphal tip, colonies were more uniform with no extreme variants unlike with the single zoospores. An important finding was that when Caten and Jinks (1968) compared the growth rate of parental isolates and the zoospore progenies, the third progeny generation had greater variation in growth rate than the earlier progenies. The variation in the progeny was relative to the parental isolate, for example a slow growing isolate would give rise to slow growing progeny, but there would be variation between them. In relation to genotype 13_A2, an aggressive parental genotype would give rise to aggressive progeny but there would be variation between them, as seen in this study. In Caten and Jinks's (1970) study on variation in the aggressiveness and virulence of single zoospores derived from three

isolates, all three parents were categorised as aggressive, but out of all the single zoospores only 25% were categorised as aggressive and 31% were categorised as not aggressive. Some even appeared to have lost pathogenicity all together. Such extensive variation within an isolate could be a method of generating different variants within the genotype. Samen *et al*, (2003a) went further and suggested that the amount of sporangial variation depended upon the isolate and considerable inherent genetic variation was found amongst asexual populations (Samen *et al*., 2003b). Variation in other *Phytophthora* has also been documented and is review by Erwin (1983).

3.4.4 Conclusion

Genotype 13_A2 is not the most aggressive *P. infestans* genotype on foliage at 15°C when compared to other contemporary UK genotypes. When assessing foliar aggressiveness only one part of the life cycle is taken into account and only a single cycle within that; to understand the reasons behind the dominance of genotype 13_A2it may be necessary to study its behaviour over successive life cycles.

Chapter 4 – Competition between *P. infestans* genotypes

4.1 Introduction

Competition between genotypes may be a factor that has contributed to the dominance of genotype 13_A2 in the UK population. The recent changes in the UK population have given rise to new genotypes that are fitter than the 'old' genotypes. The 'old' genotypes refers to those that were in the population before the discovery of the A2 mating type in Europe, the 'new' genotypes refers to the new types that were discovered after the introduction of the A2 population, but comprise both A2 and A1 mating types (Spielman *et al.*, 1991). An increase in competitive fitness of the genotypes was seen in the US. Miller and Johnson (2000) concentrated on determining the differences in competitive ability between the old population (US-1) and the new populations (US-8). Pre-infected plants were introduced into an experimental plot and the plots were checked twice a week for disease. Miller and Johnson (2000) found that US-8 was fitter than US-1; it was the dominant genotype isolated from the population, with only a few US-1 isolates being recovered. The lesion expansion of US-8 was significantly larger than that of US-1; this could affect infection by US-1, as US-8 would be colonising more parts of the plant, leaving fewer healthy tissues for US-1 to infect. Young *et al.* (2009) looked at the effect differing cultivars had on the competitive ability of genotypes and found that US-8 always dominated the population. Miller and Johnson (2000) also suggested that if the sporangia of isolates of US-8 germinated faster than those of US-1, this would allow US-8 to infect more frequently than US-1. The isolates from both genotypes were capable of causing disease, but it seemed that once US-8 was established the other genotype had trouble infecting. Competition between genotypes could also be a result of the infection process. One genotype could overcome more R genes making it a better competitor, or once an infection by one genotype has taken place other infections are inhibited.

4.1.1 UK population

In the UK, genotype 13_A2 dominated the populations between 2005 and 2010. Cooke *et al*. (2012a) conducted a field trial in May 2007 to look at competition and aggressiveness of *P. infestans* genotypes from the UK population. A randomised complete block design consisting of four blocks with four plots for each potato cultivar (Lady Balfour, Cara, Estima, Maris Piper and King Edward) was used. A mixed sporangial suspension containing isolates of the genotypes 13_A2, 2_A1, 6_A1, 7_A1 and 8_A1 was used to spray inoculate the lower leaves of the central plant in all plots. Irrigation was applied in the morning and afternoon throughout the epidemic. Infection had spread from the central plants 11 days post inoculation and samples were taken over a three week period from plants that had visible sporulating single lesions. Genotyping showed that 90% of the samples taken were of the genotype 13_A2; it had out-competed all other genotypes that were present in the sporangial mix and it was also capable of infecting the guard plants of cultivar Stirling which had a resistance rating of 8 at the time (Cooke *et al*., 2010). Thus genotype 13_A2 had a significant competitive ability over other UK genotypes on all five cultivars tested. In Chapter 3, it was shown that at 15°C genotype 13_A2 was not the most aggressive genotype when compared to 10 other UK genotypes. Aggressiveness of a genotype does not fully explain the dominance; therefore some other mechanism must drive dominance.

4.1.2 Direct competitive interaction

Young *et al*. (2009) stated that a direct competitive interaction must be occurring if aggressiveness does not account for dominance (Young *et al*., 2009). There could be inhibitory effects that would give one genotype a competitive advantage over other genotypes (Young *et al*., 2009). The activation and suppression of different defence responses is dependent on the genotype and the cultivar. For example, Wang *et al*. (2004; 2005; 2006; 2008) assessed the levels and accumulation of defence response

transcripts during infection of US-1 and US-8 on two cultivars, Russet Burbank and Kennebec. US-8 was proven to be more aggressive than US-1 on both cultivars and HR (discussed in Chapter 1.11.1) was produced earlier and more frequently with US-1 than US-8 (Wang, 2008). During the infection with US-1, PAL, HMG (defence related genes encoding for phenylalanine ammonia lyase and 3-hydroxy-3-methylglutaryl co enzyme A reductase) (Wang *et al.*, 2004), PR-1 and PR-5 (Wang *et al.*, 2005) increased in both cultivars (Wang *et al.*, 2004). The accumulation of PR-1 and PR-5 happened locally and proximally after 24-72 hours, but there was little accumulation distally (Wang *et al.*, 2006). Infection by US-8 generally produced a weaker and slower accumulation of the transcripts compared to US-1 (Wang *et al.*, 2004; Wang *et al.*, 2005). This could suggest that when *P. infestans* infects it inhibits defence responses but does not affect other defence genes which will stop other isolates infecting in close proximity. This could be an important factor for aggressive genotypes, if one genotype stops the infection of another and produces large lesions faster than the other genotype, more sporangia may be produced, giving the aggressive genotype an advantage to infect the next leaf or plant. Cultivar had an effect on the accumulation of the transcripts. Kennebec gave a stronger and quicker induction of the transcripts compared to Russet Burbank. Kennebec contains R1 and Wang *et al*. (2005) suggest it has a mechanism that enables it to respond earlier to infection. Another possible cause of one genotype being more competitive could be due to an RNA symbiont being present. Judelson *et al*. (2010) found that the RNA symbiont PiERE (*P. infestans* extrachromosomal RNA element) makes an isolate have a higher optimal growth temperature than an isolate lacking PiERE. This could be important because in the population isolates with PiERE could survive at higher temperatures whereas others would not.

4.1.3 Aim

The aim of this study was to investigate the competitive ability of four UK *P. infestans* genotypes on two potato cultivars; Maris Piper and Cara. When 13_A2 was used in mixed inoculum by Cooke *et al.* (2012a) it out-competed all other genotypes. However, in the field it is more likely that different genotypes will be introduced from different inoculum sources. Using separate inoculum of each genotype allowed investigation of how the genotypes affected each other's disease progression by studying the disease progression within plots, the pattern of spread and the incidence of the genotypes.

4.2 Method

4.2.1 Field trial

A field trial containing two potato cultivars was planted on the 4th May 2011 at the Agri-Food and Biosciences Institute (AFBI), Belfast, Northern Ireland. Four isolates were chosen to represent each genotype (Table 4.1) and were passaged through potato cultivar Up-to-Date (susceptible) before being used to make inoculum (as described in Chapter 2.4).

4.2.1.1 Design

The field trial had a total of 24 plots; a fully randomised block design was used with four replicate blocks each containing six plots of 16 plants. A single plot consisted of four rows of four plants. There were two treatments, cultivar and inoculation treatment. In each block, there were three plots of the potato cultivar Cara and three plots of potato cultivar Maris Piper and all plots were surrounded by a guard row of the resistant potato cultivar Sárpo Mira. An unplanted drill was left between each plot. Each plot was 1.8 m in length and the whole trial was 11.7 m in total (Figure 4.1). Three inoculation treatments were used in the trial: the top left corner plant (Plant 1, Figure 4.1) of each plot was inoculated with genotype 13_A2 and the bottom right corner plant (Plant 16, Figure 4.1) was inoculated with genotype 6_A1, 7_A1 or 8_A1. Inoculation treatment and cultivars were randomised within the blocks. The day before inoculation each leaflet that was to be inoculated was labelled with a coloured tag corresponding to the genotype inoculating that leaf.

4.2.1.2 Inoculation

Inoculation took place on the 29th June 2011. The method of inoculum production was previously described in Chapter 2.4. Individual tagged leaves were inoculated by placing drops of inoculum on the leaflet surfaces using a plastic disposable pipette;

approximately 3 ml of inoculum were used to inoculate each leaf. The inoculum for each genotype contained four isolates of that genotype (Table 4.1). The leaf was then enclosed in a clear plastic bag, tied around the leaf stalk to ensure high humidity during infection. The bags were removed after 24 hours.

4.2.1.3 Assessments and analysis

Field trial sampling was described in Chapter 2.6 and the method of monitoring and disease scoring was described in Chapter 2.8. ANOVAs were conducted on the AUDPC (calculation described in Chapter 2.8) values for 14 days post inoculation to 35 days post inoculation. Only scores between these days were used because all plants had infection from 14 days onwards and 35 days post inoculation was the last date on which all plants were scored. Genstat 13th Edition was used to conduct statistical analyses.

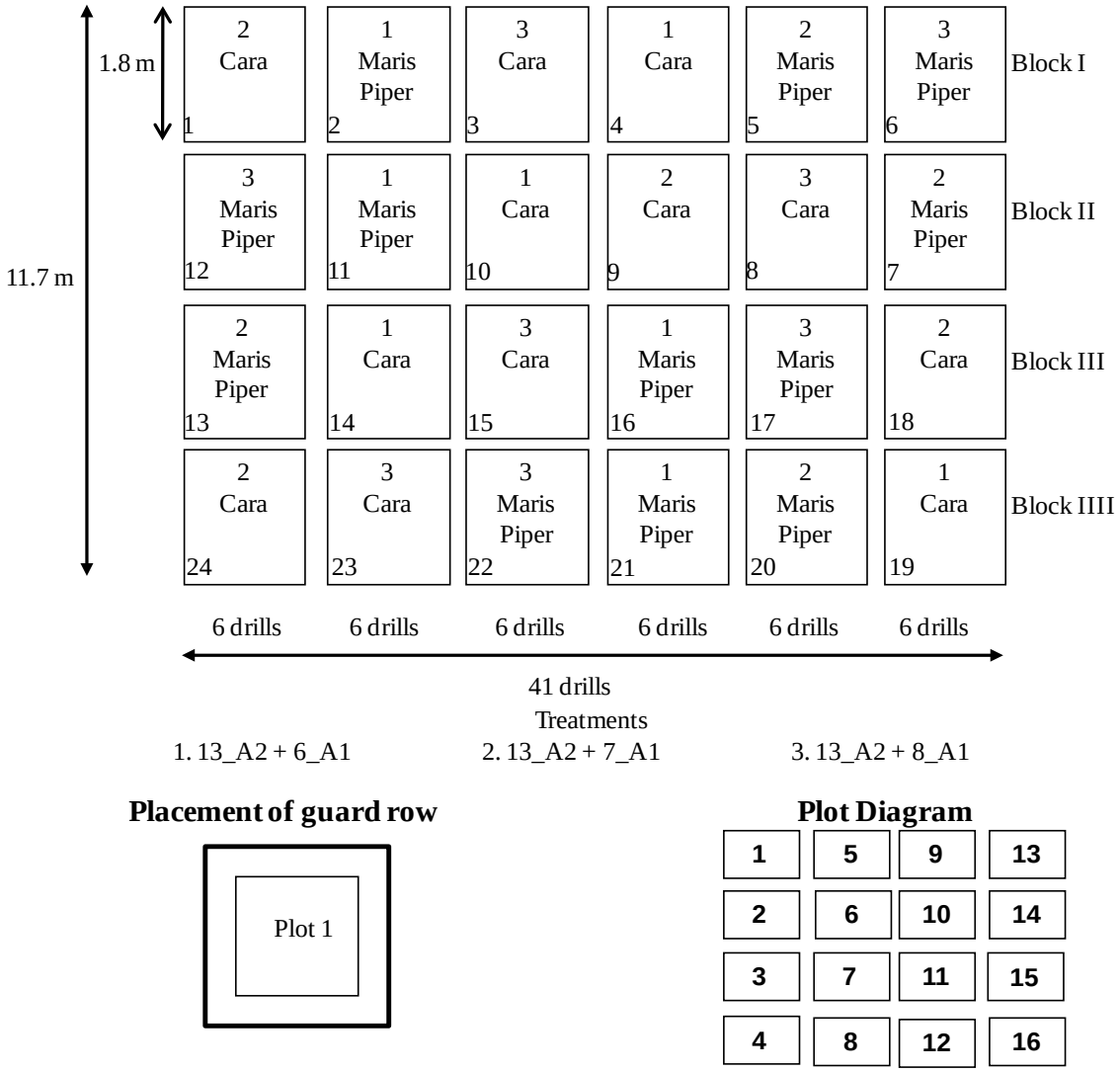


Figure 4.1 – Plot design for competition field trial in AFBI, Belfast, NI in 2011. Three inoculation treatments all including genotype 13_A2 challenged with an A1 genotype were used to infect potato cultivars Cara and Maris Piper to assess the competitive ability and epidemic spread

Thick line shows the placement of the guard row (resistant cultivar Sapro Mira). All plots had a guard row

Plot diagram shows the layout of a single plot within the trial with each box representing a plant

Table 4.1 – List of isolates used to represent each genotype in the field trial

Genotype 13_A2	Genotype 6_A1	Genotype 8_A1	Genotype 7_A1
2006_3928A (13_A2_1)	2008_6090A	2006_4168B	2006_4232E
07_39 (13_A2_5)	2008_6306A	2007_5290C	2008_6222A
2008_6102A (13_A2_2)	2008_6426A	2007_5918A	2008_6422F
2008_6194A (13_A2_1)	2008_6502A	2008_6274D	2008_6458E

4.2.2 Laboratory assay

A whole detached leaf assay was used to investigate the effect of a primary infection by an isolate of a UK *P. infestans* genotype upon a secondary infection of an isolate of a different, or the same genotype. One isolate was chosen to represent each genotype as it would be hard to distinguish the effects that within-genotype competition had on the results if mixed inoculum was used. Genotypes were the same as those used in the field trial (Figure 4.2.B). Isolates were passaged on Craig's Royal before use (Chapter 2.4) and the genotypes were tested on Maris Piper whole detached leaflets.

4.2.2.1 Design

Fifty-four plastic boxes with sealable lids (52 x 34 x 8.5 cm) were lined with damp paper towelling and two whole detached leaflets were placed in each box abaxial side up. In each box, one treatment at each of two locations (Near and Far, Figure 4.2.C) was tested. A fully randomised block design was used with three replicate blocks and each of the 18 treatments were randomised within the blocks.

4.2.2.2 Inoculum and incubation

Inoculum preparation was described in Chapter 2.4. Firstly, a 15 µl droplet of the primary sporangial suspension was placed on to the leaflet near the midrib on the abaxial surface and in the same position on each leaflet. The secondary inoculation was done 2 days after the primary inoculation as this gave enough time for a primary infection to establish. Table 4.2 shows the different combinations of treatments that were used. After each inoculation, the boxes were placed in clear plastic bags to maintain humidity and then covered with tissue for 24 hours. The boxes were stored in a walk-in growth chamber (Reftech walk-in growth chamber) at 15°C with a 16 hour/8 hour light/dark cycle.

4.2.2.3 Data collection and analysis

Aggressiveness criteria were recorded for 7 days. IP and LP were determined by eye. The lesion size, for both primary and secondary inoculations, was measured 7 days post inoculation and growth rate per day was used in the analysis of variance (ANOVA).

Table 4.2 – Treatment combinations for the laboratory assay on whole detached leaflets of potato cultivar Maris Piper

Treatment	Primary Inoculation	Secondary Inoculation
1	13_A2	6_A1
2	6_A1	13_A2
3	13_A2	8_A1
4	8_A1	13_A2
5	13_A2	7_A1
6	7_A1	13_A2
7	13_A2	Water
8	6_A1	Water
9	8_A1	Water
10	7_A1	Water
11	Water	13_A2
12	Water	6_A1
13	Water	8_A1
14	Water	7_A1
15	13_A2	13_A2
16	6_A1	6_A1
17	7_A1	7_A1
18	8_A1	8_A1

Table 4.3 – List of the isolates that were used to represent the genotypes used in the assay

Genotype	Isolate
13_A2	2006_3928A
6_A1	2008_6090A
8_A1	2006_4232E
7_A1	2008_6274D

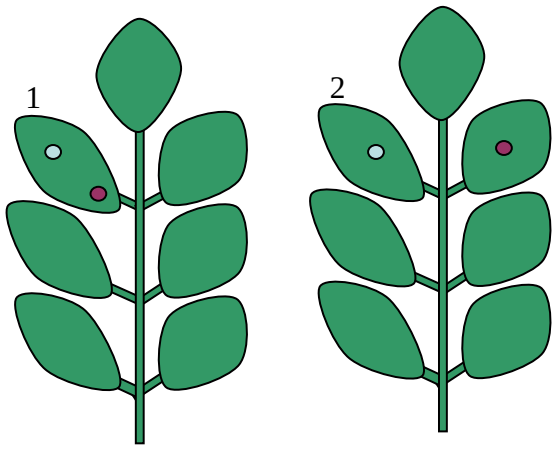


Figure 4.2 – Diagram of the primary and secondary inoculation points on Maris Piper whole detached leaflets

1) Near – both inoculation on the same leaflet

2) Far – secondary inoculation on adjacent leaflet to primary inoculation

4.3 Results

4.3.1 Field trial

4.3.1.1 Weather data

The average temperature ranged from 12.4°C to 17.3°C and the average temperature over all days was 14.6°C (Figure 4.3). During the first four days after inoculation the average temperature ranged from 12.4°C to 13.8°C. The RH ranged from 63% to 89% and over all the days the average RH was 77%. The weather data showed that minimum temperature dipped below 10°C on 16 days of the 37 days of the epidemic. RH barely reached 90%, this only occurred on three days. Only two Smith Periods occurred during the epidemic. Irrigation was used throughout the epidemic as large amounts of rainfall (over 10 mm) only occurred twice during the epidemic.

4.3.1.2 Disease progression over time

The first visible signs of infection were seen 5 days post inoculation; this was true for all genotypes but not for all plots, and the final assessments were made 62 days post inoculation. On the final date of scoring the overall mean percentage foliar blight for all plots was 92.9%. Disease increased steadily from 14 to 19 days post inoculation and then at a faster rate from 23 to 36 days post inoculation. Scores at 50 and 62 days post inoculation show the end of the epidemic and were not included in the analysis as assessment for disease was based on rows of plants rather than individual plants (Figure 4.3.A).

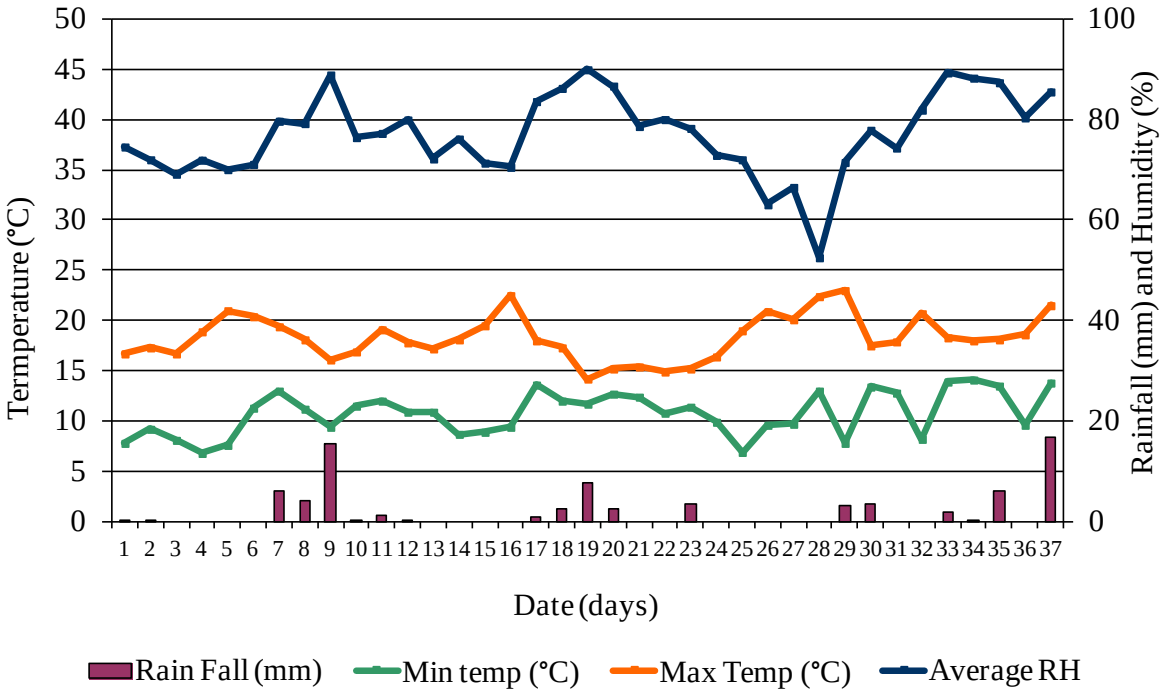


Figure 4.3 – Mean weather data for field trial in Agri-Food and Biosciences Institute (AFBI), Belfast, Northern Ireland in 2011.

4.3.1.3 Cultivar

Cultivar had a significant effect on percentage foliar blight (P<0.001, Table 4.5). Substantially more foliar blight was present on Maris Piper than on Cara for the majority of the epidemic (Figure 4.4.B). Twenty three days post inoculation there was a significant difference between the foliar blight present on each cultivar. On cultivar Cara, the increase in foliar blight from 23 to 36 days post inoculation was from 10.3% to 12.3%, whereas on Maris Piper the increase was from 17.7% to 35.0%; by 36 days after inoculation the amount of foliage infection on Maris Piper was nearly triple the amount on Cara (Figure 4.4.B). The amount of foliar blight on Maris Piper after 50 and 62 days post inoculation (96.1% and 99.6% respectively) showed that it was reaching the plateau where there was not much crop left to colonise. However on Cara, the foliar blight present after 50 and 62 days post inoculation was 51.1% and 86.1%; the epidemic on Cara lasted a longer period of time than on Maris Piper as it is more resistant.

The mean AUDPC values showed that Maris Piper had significantly more disease than Cara (P<.001, Figure 4.5.A, Table 4.5). *P. infestans* genotype combinations did not affect the amount of disease on the two cultivars (P=0.504, Figure 4.5.B, Table 4.5). Cara had similar AUDPC values for all treatments; 172.4, 152.1 and 169.8 for treatments 13_A2+6_A1, 13_A2+7_A1 and 13_A2+8_A1 respectively. Although no significant differences were seen in the AUDPC values between the inoculation treatments for Maris Piper, there was a lot more disease present for the treatment 13_A2+6_A1 (397.5) compared to 13_A2+7_A1 (293.2) and 13_A2+8_A1 (277.2). Heat plots are a graphical representation of the data that show individual values at each point in the plot and use colour to represent value ranges. The heat plots (Figure 4.5.D) show the mean AUDPC values for each plant in the plot; these clearly showed the difference between the two cultivars and the extent of the spread with the plots of each cultivar. For example, in Cara, disease was present on all plants but the three most

severely diseased plants were those that were inoculated or adjacent to the inoculated plants. Conversely, on Maris Piper all plants were severely infected.

4.3.1.4 Inoculation treatment

Inoculation treatments did not significantly affect the percentage foliar blight over the course of the epidemic (P=0.316, Table 4.5). All foliar blight values for the different genotype combinations were very similar to each other for each date except at 36 days post inoculation where treatment 13_A2+6_A1 had 28.2% foliar blight compared to 21.7% and 21.2% foliar blight for treatments 13_A2+7_A1 and 13_A2+8_A1 respectively.

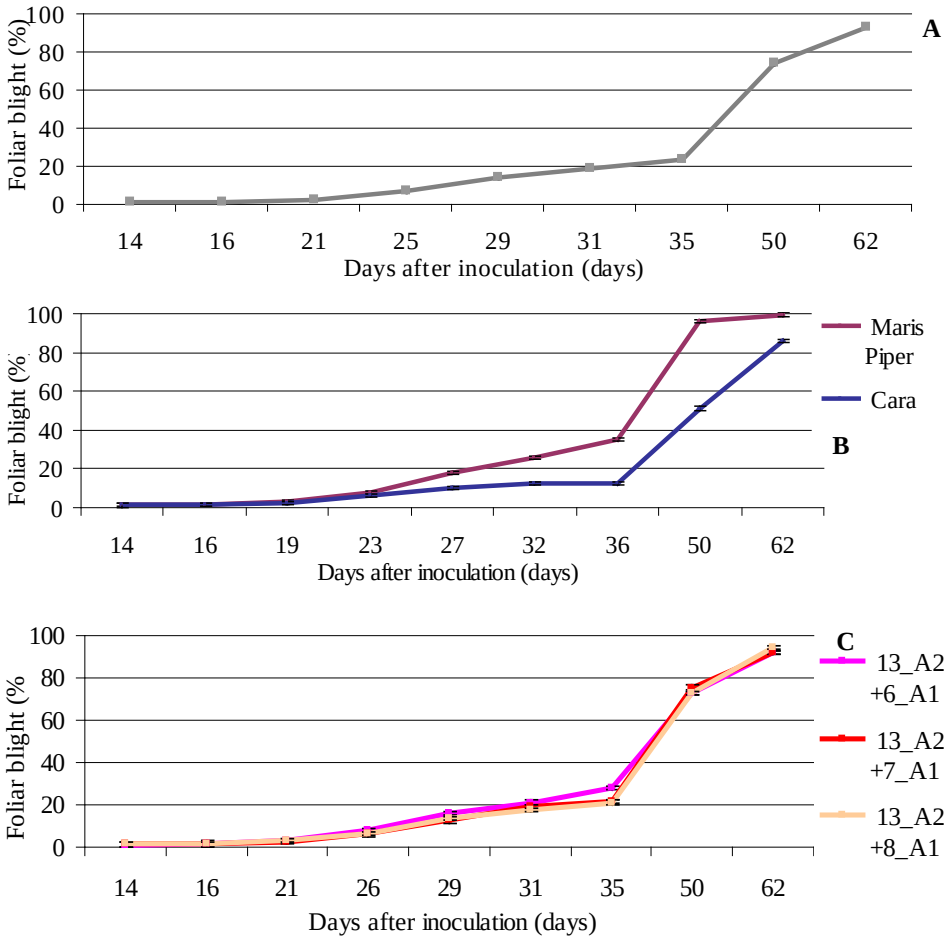


Figure 4.4 – Mean foliar blight (%) for UK *P. infestans* genotypes in a field trial on two potato cultivars. The error bars represent the standard errors of the difference of the means

A) Foliar blight (%) meaned over cultivar and treatment. SE=0.47

B) Foliar blight (%) meaned over genotype and treatment. SE=1.41

C) Foliar blight (%) meaned over cultivar. SE=1.73

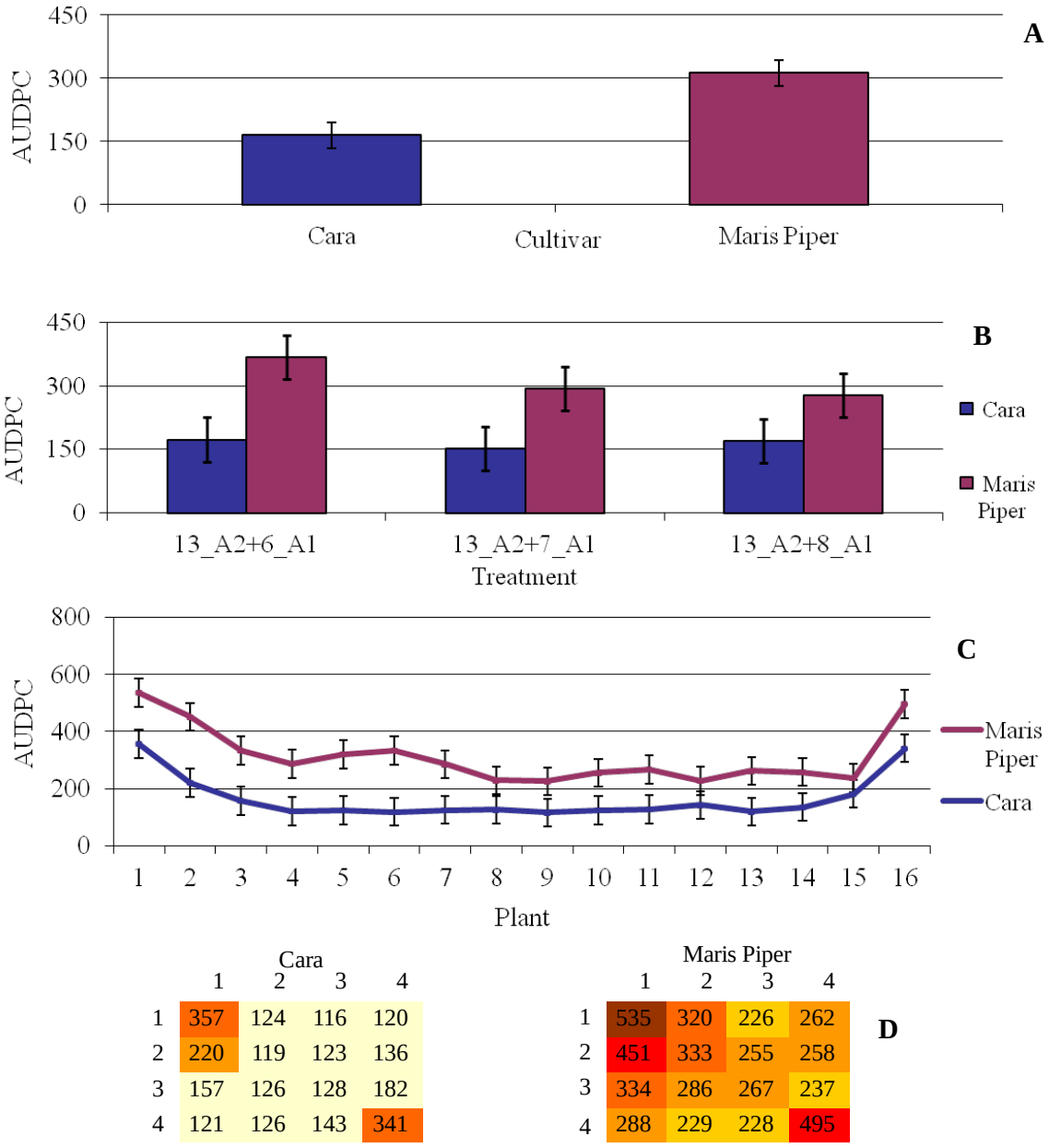


Figure 4.5 – Mean AUDPC values for UK *P. infestans* genotypes in a field trial on two potato cultivars. The error bars represent the standard errors of the difference of the means (SE)

A) Mean AUDPC value for potato cultivar (meaned over treatment) for all plots. SE=30.25

B) Mean AUDPC value for potato cultivar at each treatment over all plots. SE=52.40

C) Mean AUDPC value for plant (meaned over treatment) over all plots. SE=49.22

D) Heat plots showing the mean AUDPC value for each plant in the plot on potato cultivars Cara and Maris Piper. The colour scale used in both heat plots are the same

4.3.1.5 Inoculation treatment

The inoculation treatments had no significant effect on the AUDPC values (P=0.365, Figure 4.6.A, Table 4.5). The amount of disease caused by treatments 13_A2+7_A1 and 13_A1+8_A1 was similar over all plots containing that treatment. Although the amount of disease caused by treatment 13_A2+6_A1 was not significantly different from the other two, it did produce a larger AUDPC value of 270.0. However, when the AUDPC values for each individual plant were compared, there was no significant effect of treatment on the AUDPC values for any individual plant except of plant 16 for the treatment 13_A2+6_A1; plant 16 of the treatment 13_A2+6_A1 had an AUDPC value of 644.5 which is substantially larger than the plant 16 of the other treatments (P<0.001, Figure 4.6.B, Table 4.5). The heat plots (Figure 4.6.C) show the differences in the disease progression across the plots for the three treatments. For example, in treatments 13_A2+7_A1 and 13_A2_8_A1 the disease progression from plant 16 (the plant inoculated with either 7_A1 or 8_A1 depending on the treatment) shows minimal spread whereas, in the treatment 13_A2+6_A1 the progression of disease around plant 16 (inoculated with 6_A1) is more extensive. Disease progression from 13_A2 was similar in all plots.

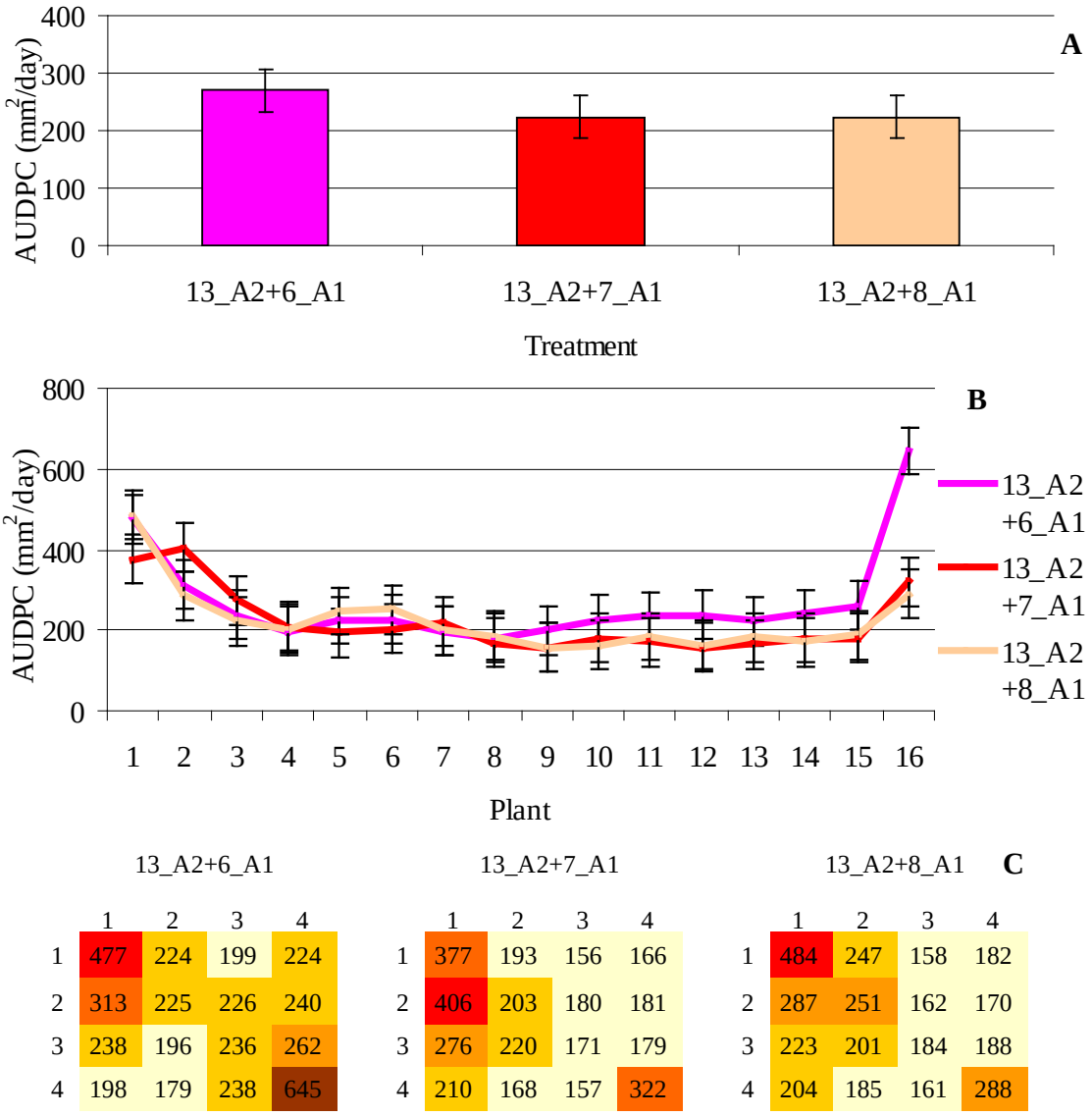


Figure 4.6 – Mean AUDPC values for UK *P. infestans* genotype in a field trial on two potato cultivars.

A) Mean AUDPC value for treatment (meaned over cultivar) for all plots. SE=37.05

B) Mean AUDPC value for treatment on plants of the plots (meaned over cultivar). SE=60.29

C) Heat plots showing the mean AUDPC value for treatments for each plant in the plot (meaned over cultivars). The colour scale used in both heat plots are the same

100-200 201-250 251-300 301-400 401-500 501+

Table 4.4 – Descriptive statistics for competition field trial: testing the competitive ability of four UK *P. infestans* genotypes on two potato cultivars

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Date	6	189920.15	31653.36	762.81	<.001
Date x Cultivar	6	43702.98	7283.83	175.53	<.001
Date x Treatment	12	3164.01	263.67	6.35	<.001

Table 4.5 – Descriptive statistics for competition field trial that assessed the competitive ability of four UK *P. infestans* genotypes on two potato cultivars of differing resistance ratings; Cara (resistant) and Maris Piper (susceptible).

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Treatment	2	187580	93790	1.07	0.365
Cultivar	1	2098642	2098642	23.88	<.001
Treatment x Cultivar	2	125027	62514	0.71	0.504
Plant	15	2582578	172172	17.84	<.001
Treatment x Plant	30	737262	24575	2.55	<.001
Cultivar x Plant	15	197785	13186	1.37	0.163

4.3.1.6 Genotyping

In total, 994 late blight lesions were collected and genotyped. Out of all the samples collected, 625 were found to be genotype 13_A2, 194 were genotype 6_A1, nine were genotype 7_A1 and eight were genotype 8_A1, showing that although sampling of 7_A1 and 8_A1 was infrequent, the inoculum was still viable. Detached leaflets were inoculated to test the viability of the sporangial/zoospore suspensions used to inoculate the plots and all suspensions were able to cause infection. A small number of samples were suspected recombinants of the genotypes used in this study (five samples), one was a mixture of two genotypes and 152 samples could not be definitively identified due to a particular marker not being amplified when the samples were genotyped. Out of the 625 genotype 13_A2 samples, 50 were identified as 13_A2 and could not be assigned to a subgroup, 428 samples were of the subgroup 13_A2_1, 147 were 13_A2_2 and there were no 13_A2_5 sampled. Genotype 13_A2 had the largest percentage of samples collected; it contributed 50% of the samples (this percentage has been weighted to account for the fact that there was 3 times more genotype 13_A2 inoculum introduced into the field compared to the other genotypes). Genotype 6_A1 contributed 46% of the samples, so genotype 13_A2 is only dominant by 4%. Both genotypes 7_A1 and 8_A1 contributed 2% of the samples taken.

4.3.1.6.1 Treatments

Genotype 13_A2 contributed 60.9% of the samples taken from plots inoculated with treatment 13_A2+6_A1 and 6_A1 contributed 39.1%, showing that genotype 13_A2 was the dominant genotype by approximately 20% (Figure 4.7.A). Plots inoculated with the treatments 13_A2+7_A1 and 13_A2+8_A1 were also both dominated by genotype 13_A2. Genotype 13_A2 represented 99.6% of samples from the 13_A2+7_A1 treatment and 98.7% from the 13_A2+8_A1 treatment; 7_A1 and 8_A1 only had very small proportions of 0.4% and 1.3% respectively (Figure 4.7.A).

4.3.1.6.2 Plots

Overall, it can be seen that genotype 13_A2 was found in the largest number of samples from most of the plots (Figure 4.7.B). 13_A2 was dominant in the majority of individual plots inoculated with the treatment 13_A2+6_A1. Only in two of the eight plots (plots 4 and 19) did 6_A1 out-compete 13_A2 (Figure 4.7.B). Cultivar was not associated with the dominance of 6_A1 in these two plots as plot 4 was Maris Piper and plot 19 was Cara. In most cases, except for plot 20, 7_A1 was completely out-competed by 13_A2 and the same can be said for 8_A1 except for plots 6, 8 and 15 where a few lesions sampled contained this genotype.

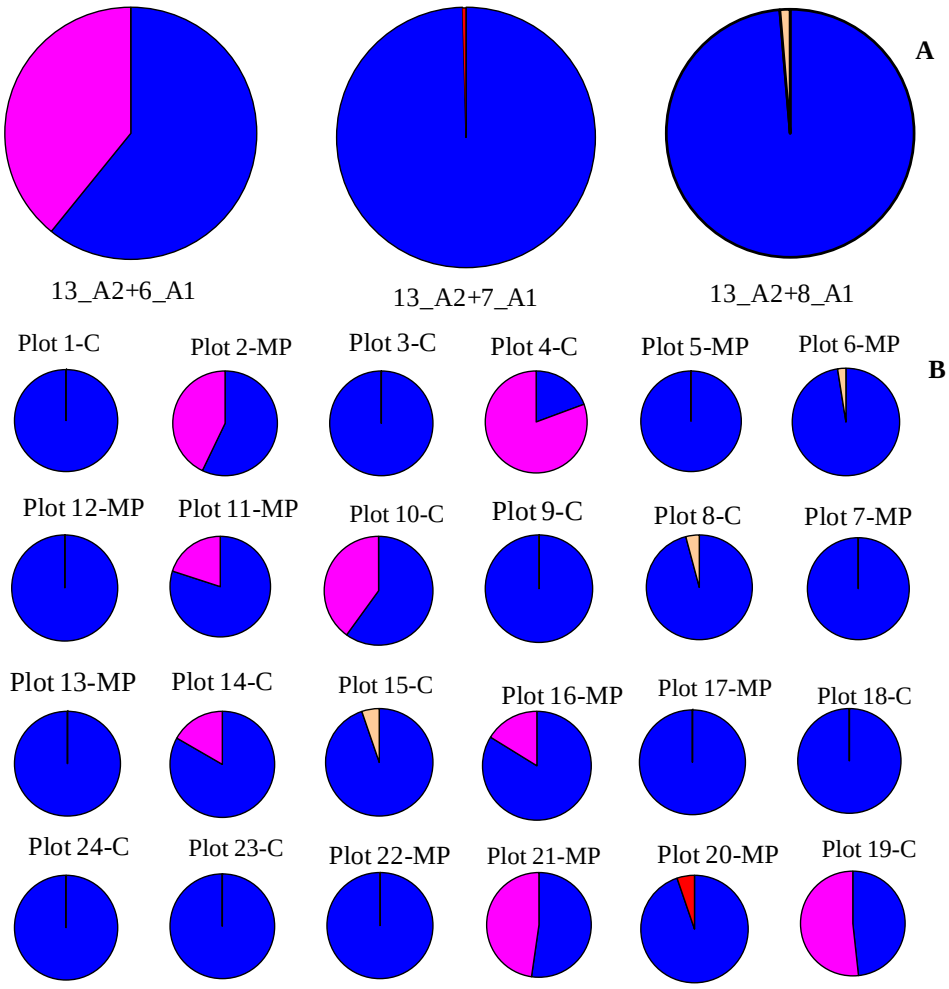


Figure 4.7 – Pie charts showing the proportion of samples collected for each of the four UK *P. infestans* genotypes in a field trial based on two potato cultivars

A) Pie chart of the proportion of samples collected for each treatment (mean of plots)

B) Pie charts of the proportion of samples taken from each plot.

Invading genotypes have been removed so proportions of the genotype belonging to the treatment are seen.

13_A2 6_A1 7_A1 8_A1

4.3.1.6.3 Disease progression within plots

Genotype 13_A2 progressed through the plots faster than the other genotypes (Figure 4.8). In some cases genotype 6_A1 had a large number of samples spread throughout the plots, showing that it is able to spread but in other cases spread within the plots was not seen; this was not related to cultivar. Samples of 8_A1 and 7_A1 were not collected in all plots. The prevailing wind across the plot was south-westerly; genotype 13_A2 samples showed that the spread direction was north-west to south-east, working against the prevailing wind.

For treatment 13_A2+6_A1, samples taken at the first sample date consisted of just genotype 13_A2 (16 days post inoculation) and the second sample date (19 days post inoculation) consisted of genotype 6_A1. After these two sample dates both genotype 13_A2 and genotype 6_A1 samples were taken at each sample date, though more genotype 13_A2 samples were taken. Both genotypes showed steady spread throughout the plot over the sample dates. On some sample dates the number of samples taken of each genotype was similar but on other sample dates the number of samples taken of genotype 13_A2 greatly outweighed that of genotype 6_A1. For example, 23 days post inoculation 13 genotype 13_A2 samples and 8 genotype 6_A1 samples were collected. Conversely, 27 days post inoculation 60 samples of genotype 13_A2 and only 32 samples of genotype 6_A1 were collected. Only at 33 days post inoculation did the number of samples taken for both genotype 13_A2 and 6_A1 match with 40 samples taken of each. After this sample date the same pattern of more genotype 13_A2 samples being taken continued. As the epidemic progressed, more samples of each genotype were taken from the plots as there were more lesions on each plant available to sample. The same pattern is not seen for treatments 13_A2+7_A1 and 13_A2+8_A1 as genotype 7_A1 and 8_A1 did not spread throughout the plot. In treatment 13_A2+7_A1, genotype 7_A1 was first sampled on the second to last sample date (33 days post

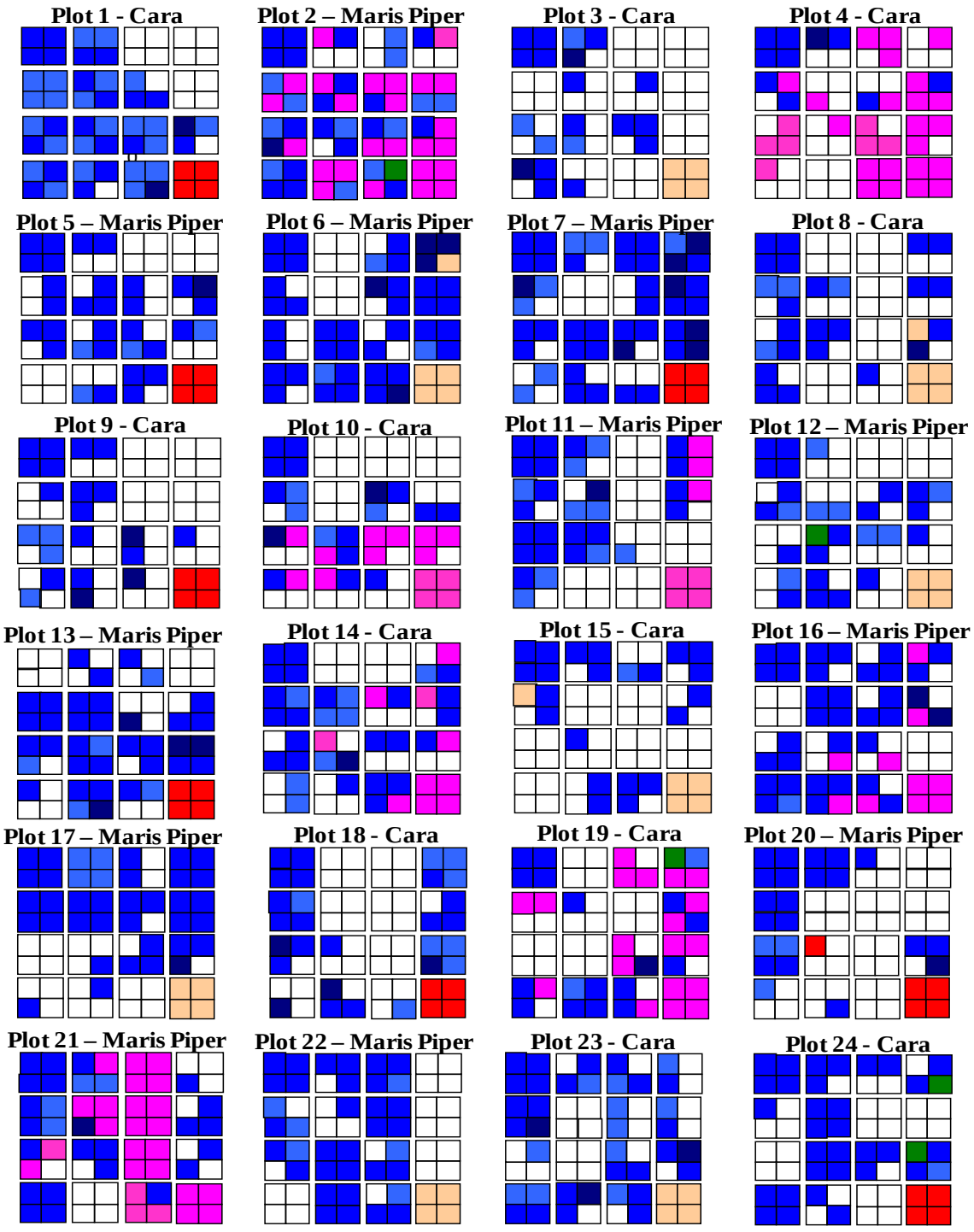
inoculation), all other samples taken at each date were genotype 13_A2. Genotype 8_A1 was sampled for the first time 23 days post inoculation and then on two more occasions at the next two sampling dates, showing a slow spread over time.

4.3.1.6.4 Spread outside of the plots

Assessing the genotypes of the samples showed that some plots had genotypes present that were not part of that particular inoculation treatment, hereafter referred to as foreign samples (not shown in Figures). For example, genotype 6_A1 was found in many plots which had not been inoculated with it; it was used to inoculate 8 out of the 24 plots, but was found in an additional 13 plots and only three plots were free from 6_A1. In the majority of cases the number of foreign samples found in the plots was small. For example, in plot 3 which was inoculated with the treatment 13_A2+8_A1, four samples of 6_A1 and one sample of 7_A1 were found. There were a few plots where a lot of foreign samples were found. For example, in plot 20 which was inoculated with 13_A2+7_A1, 17 samples of 6_A1 were found. Not all of the 7_A1 and 8_A1 samples were found in the plots inoculated with these genotypes. Out of the nine samples of 7_A1, eight were found in non 13_A2+7_A1 plots and out of the eight samples of 8_A1, five were found in non-13_A2+8_A1 plots.

It was easy to distinguish between the A1 genotypes that had escaped from the treatment plots, but for the genotype 13_A2 samples, it was impossible to distinguish between samples spread from within a plot and samples that were foreign to that plot. Concentrating on genotype 13_A2 and 6_A1, as those had the most samples present out of all four genotypes; the number of genotype 13_A2 samples that have travelled to a different plot (foreign samples) cannot be estimated as these samples cannot be distinguished by genotyping. Out of the 194 samples which contained 6_A1, 84 were foreign samples found in plots in which they were not introduced. Genotype 13_A2

was included in every treatment and therefore was represented three times more than genotype 6_A1 within the trial.. Assuming that the rate of escaping foreign samples of genotype 13_A2 was the same as the rate at which genotype 6_A1 escaped, 252 genotype 13_A2 samples could be classed as foreign samples as 84 samples (number of genotype 6_A1) multiplied by three is 252. These samples may not have been classed as foreign had it been possible to determine which sub-genotype of 13_A2 was present.. Even with the estimated number of foreign samples subtracted from the total samples, genotype 13_A2 was still dominant and made up 52% of the samples, while genotype 6_A1 made up 46% of the samples.



Plot Diagram

1	5	9	13
2	6	10	14
3	7	11	15
4	8	12	16

Figure 4.8 – Plot diagrams showing where each genotype was found during sampling. Each plant is represented by four blocks as four samples were taken from each plant. Final sample date shown

Invading genotypes were taken out of the plots so just the correct genotypes can be seen.

13_A2 13_A2_1 13_A2_2 6_A1

7_A1 8_A1 Recombination/Mixture

4.3.2 Laboratory assay

4.3.2.1 Incubation period

In both the ‘Near’ and ‘Far’ leaf locations, significant differences were found between the IP values of the primary inoculation site and the secondary inoculation site (Near $P<0.001$, Figure 4.9.A, Table 4.6, Far $P<0.001$, Figure 4.10.A, Table 4.7). The primary inoculation inhibited the secondary inoculation, increasing the IP values.

Concentrating on the secondary inoculation at the Near inoculation site, there were no significant differences in the IP values ($P=0.291$, Figure 4.9.B, Table 4.6). Genotypes 13_A2, 6_A1 and 8_A1 had similar IP values when they were challenged with another genotype (either the same or a different genotype) or with water. Genotype 7_A1 took longer to infect when the primary inoculation was genotype 13_A2 or 7_A1 itself compared to when the primary inoculation was water, showing that the primary inoculation did have a significant effect on the IP for genotype 7_A1. The same pattern was seen at the far inoculation site ($P=0.507$, Figure 4.10.B, Table 4.7)

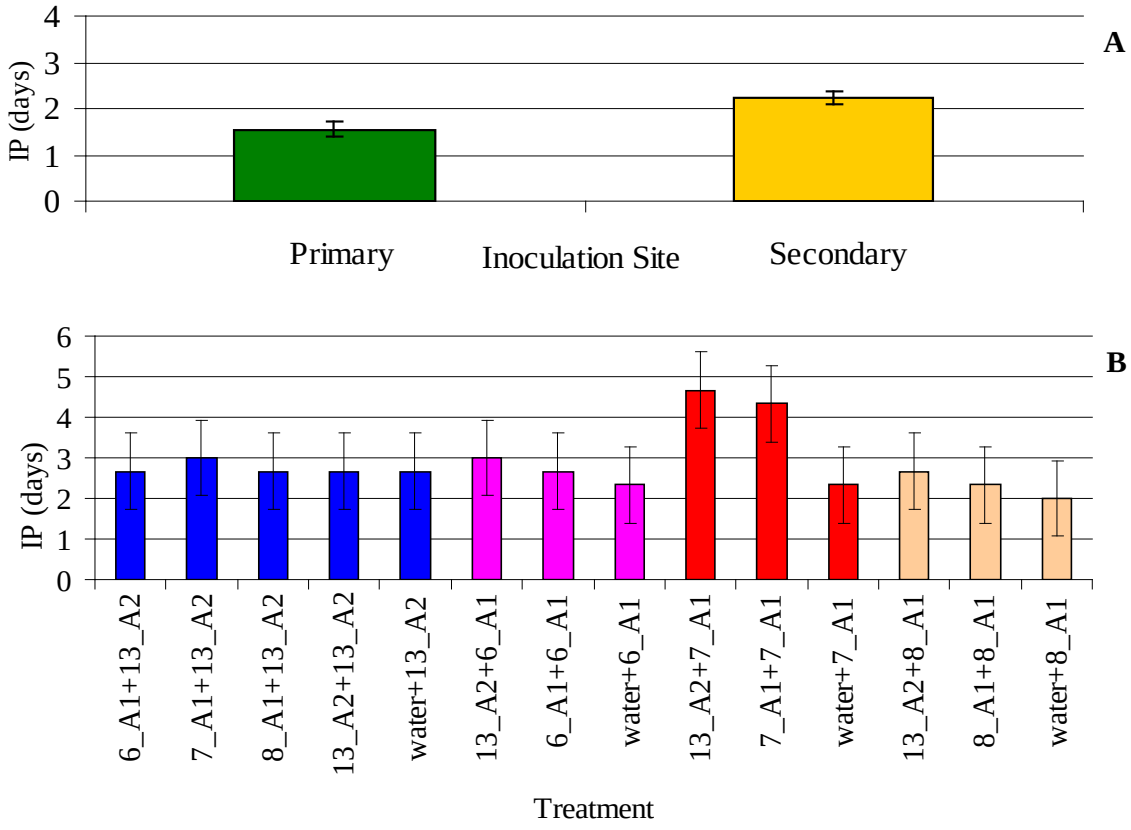


Figure 4.9 – Mean IP values for four UK *P. infestans* genotypes on whole detached potato leaflets representing the Near category.

A) Mean IP value for each inoculation site (meaned over treatment, water control included in analysis). SE=0.15

B) Mean IP values for each treatment (water control at the secondary inoculation site not shown or included in analysis) at the secondary inoculation site. Colour of bars represent the genotype that was used as the secondary inoculation. SE=0.94

13_A2 as secondary inoculation

6_A1 as secondary inoculation

7_A1 as secondary inoculation

8_A1 as secondary inoculation

Table 4.6 – Descriptive statistics for IP values for the laboratory assay assessing the competitive ability of four UK *P. infestans* genotypes on whole detached leaflets at the Near inoculation site

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Inoculation Site	17	70.9	4.17	6.35	<.001
Treatment	13	21.81	1.67	1.27	0.291

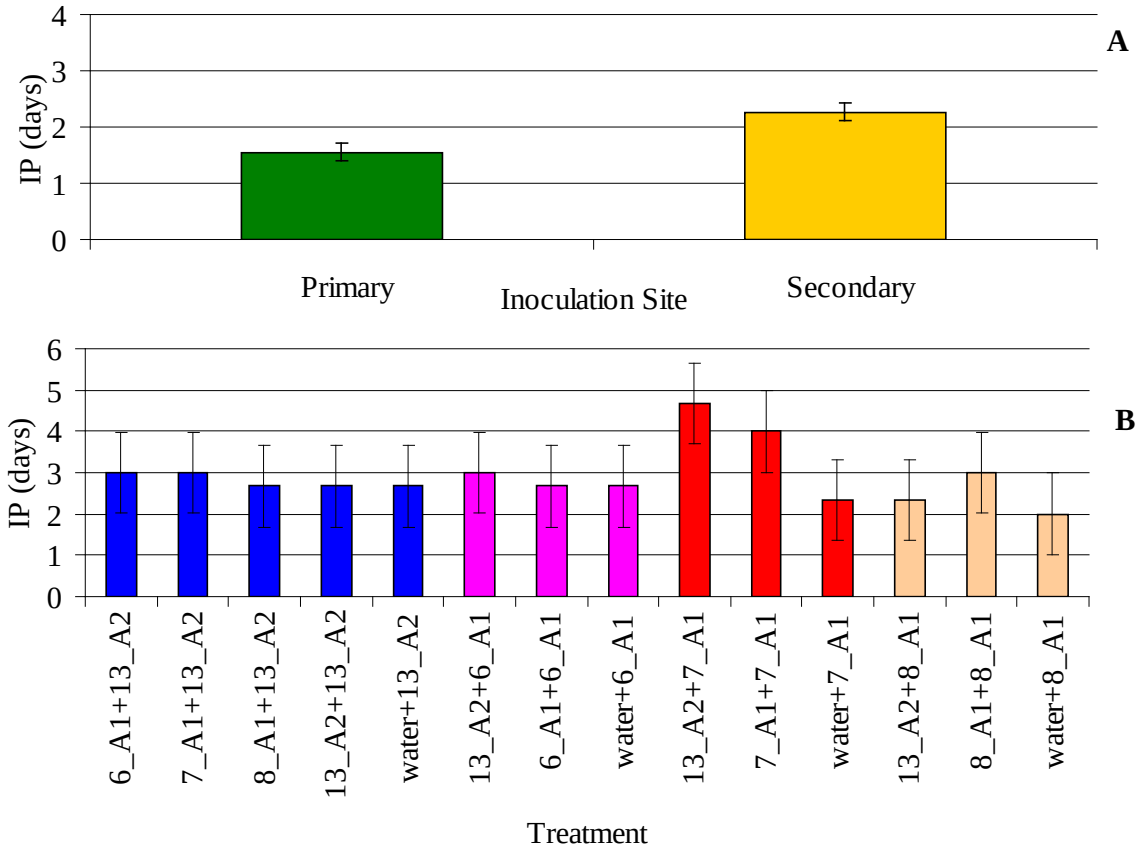


Figure 4.10 – Mean IP values for four UK *P. infestans* genotypes on whole detached potato leaflet representing the Far category. The error bars represent the standard error of the difference of the means

A) Mean IP values of each inoculation site (meaned over treatment). SE=0.16

B) Mean IP values for each treatment (water control at the secondary inoculation site not shown or included in analysis) at the secondary inoculation site. Colour of bars represent the genotype that was used as the secondary inoculation. SE=0.99

13_A2 as secondary inoculation

6_A1 as secondary inoculation

7_A1 as secondary inoculation

8_A1 as secondary inoculation

Table 4.7 – Descriptive statistics for IP values for the laboratory assay assessing the competitive ability of four UK *P. infestans* genotypes on whole detached leaflets at the Far inoculation site

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Inoculation Site	17	70.9	4.17	6.35	<.001
Treatment	13	18.28	1.40	0.97	0.507

4.3.2.2 Latent period

For the Near inoculation, the primary site had a significantly longer LP than the secondary site according to the mean values of the LP for the Near and Far locations (Near $P<.001$, Figure 4.11.A, Table 4.8). Conversely, for the Far location, the secondary site LP value was longer than that at the primary site (Far $P<.001$, Figure 4.12.A, Table 4.9).

Comparing the LP values of the Near location for the secondary inoculations, there were no significant differences between the genotypes ($P=0.455$, Figure 4.11.B, Table 4.8). The secondary inoculation LP values of each of the genotypes 13_A2, 6_A1 and 7_A1 were not significantly different from each other when paired with a different primary inoculation; for example, the LP values for genotype 13_A2 did not differ significantly whether it was paired with a different genotype, the same genotype or water. However, a difference in LP values was seen when genotype 8_A1 was the secondary inoculation. The LP values were the same when genotype 8_A1 was paired with 13_A2 and water, but when it was paired with 8_A1 (the same genotype) the LP value for the secondary inoculation was significantly longer. The same pattern was seen for the secondary inoculations at the Far location for genotypes 6_A1, 7_A1 and 8_A1, but not for 13_A2 ($P=0.301$, Figure 4.12.B, Table 4.9).

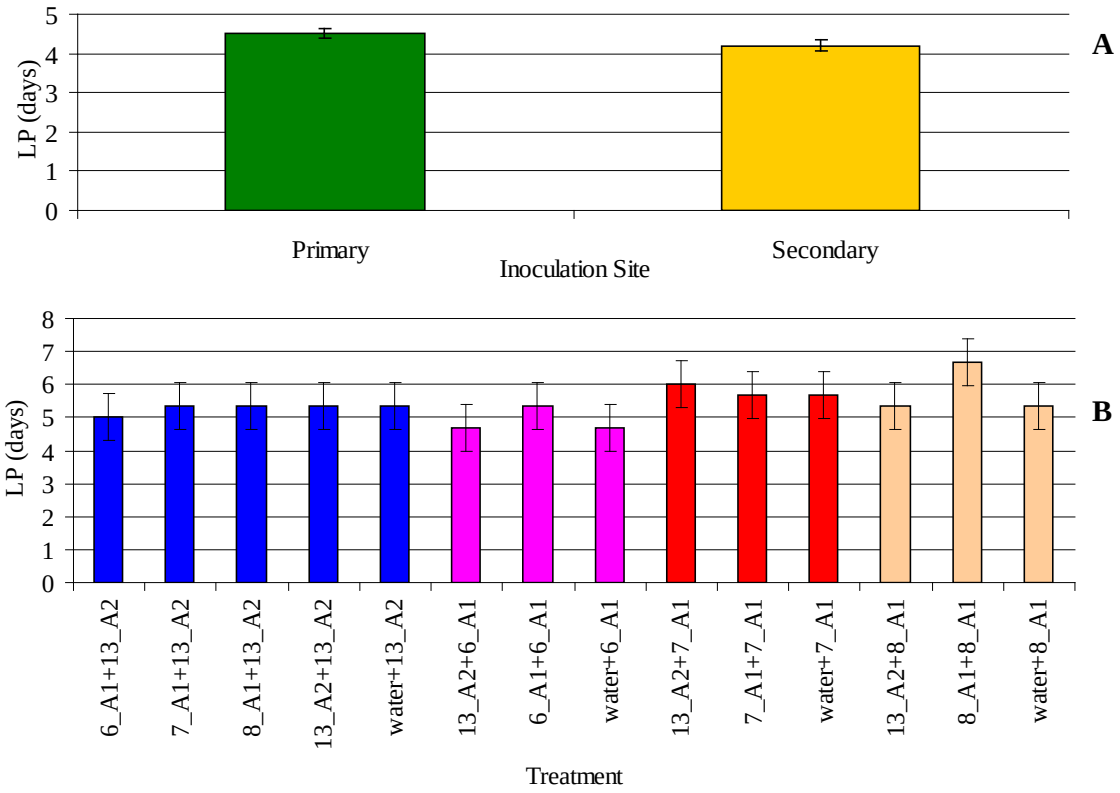


Figure 4.11 – Mean LP values for four UK *P. infestans* genotypes on whole detached potato leaflets representing the Near category. The error bars represent the standard error of the difference of the means

A) Mean LP values of each inoculation site (meaned over treatment). SE=0.13

B) Mean LP values for each treatment (water control at the secondary inoculation site not shown or included in analysis) at the secondary inoculation site. Colour of bars represent the genotype that was used as the secondary inoculation. SE=0.71

13_A2 as secondary inoculation

6_A1 as secondary inoculation

7_A1 as secondary inoculation

8_A1 as secondary inoculation

Table 4.8 – Descriptive statistics for LP values for the laboratory assay assessing the competitive ability of four UK *P. infestans* genotypes on whole detached leaflets at the Near inoculation site

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Inoculation Site	17	400.96	23.58	51.99	<.001
Treatment	13	10.19	0.77	1.03	0.45

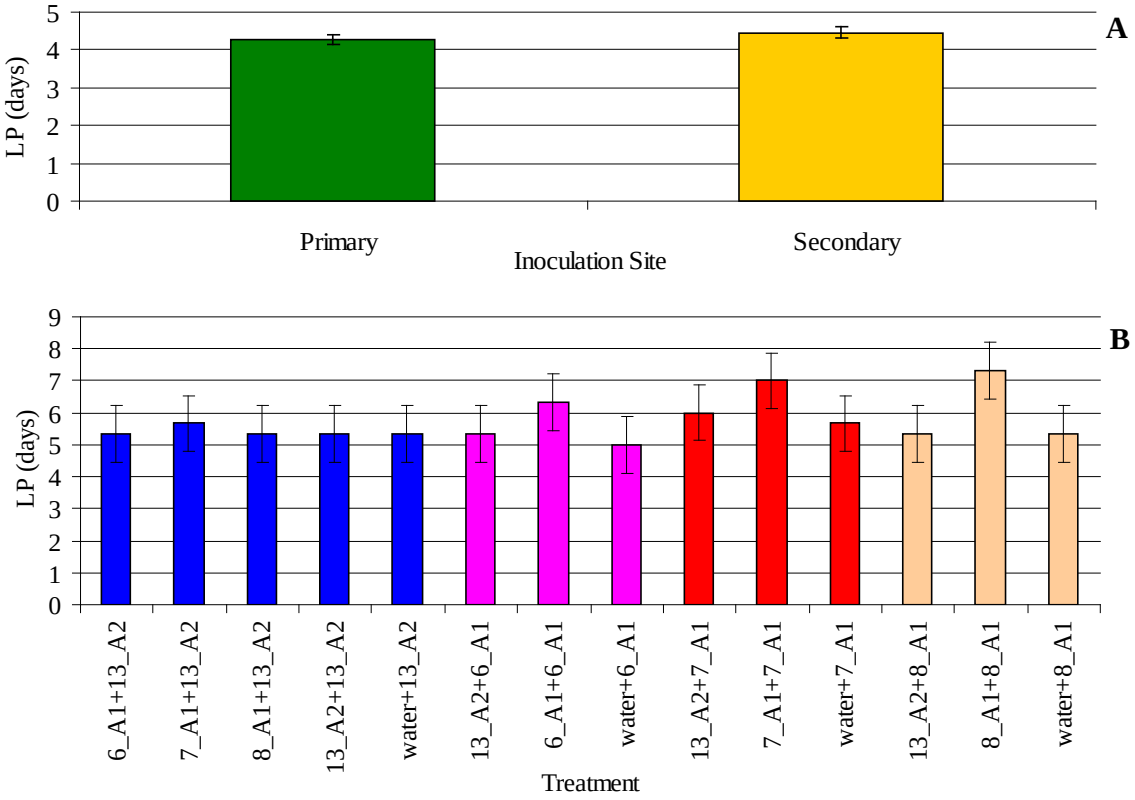


Figure 4.12 – Mean LP values for four UK *P. infestans* genotypes one whole detached potato leaflets representing the Far category. The error bars represent the standard error of the difference of the means

A) Mean LP values for each inoculation site (meaned over treatment). SE=0.14

B) Mean LP values for each treatment (water control at the secondary inoculation site not shown or included in analysis) at the secondary inoculation site. Colour of bars represent the genotype that was used as the secondary inoculation. SE=0.88

13_A2 as secondary inoculation

6_A1 as secondary inoculation

7_A1 as secondary inoculation

8_A1 as secondary inoculation

Table 4.9 – Descriptive statistics for LP values for the laboratory assay assessing the competitive ability of four UK *P. infestans* genotypes on whole detached leaflets at the Far inoculation site

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Inoculation Site	17	397.40	23.37	41.39	<.001
Treatment	13	18.786	1.44	1.25	0.301

4.3.2.3 Lesion expansion

The growth rate of the lesion at the primary inoculation site was significantly greater than that at the secondary inoculation sites in both the Near and Far locations showing that the primary inoculation affected the growth of a secondary inoculation (Near $P<0.001$, Figure 4.13.A, Table 4.10, Far $P<0.001$, Figure 4.14.A, Table 4.11).

At the Near site, genotype 13_A2 showed no drastic changes in lesion size regardless of what it was paired with. It did have a larger lesion size when paired with water but the differences were not significant. Both 7_A1 and 8_A1 had a very much smaller lesion size when paired with genotype 13_A2 than when these genotypes were paired with either themselves or water. The same patterns were seen for the Far site, except that this time, genotype 6_A1 also had a smaller lesion size when paired with genotype 13_A2.

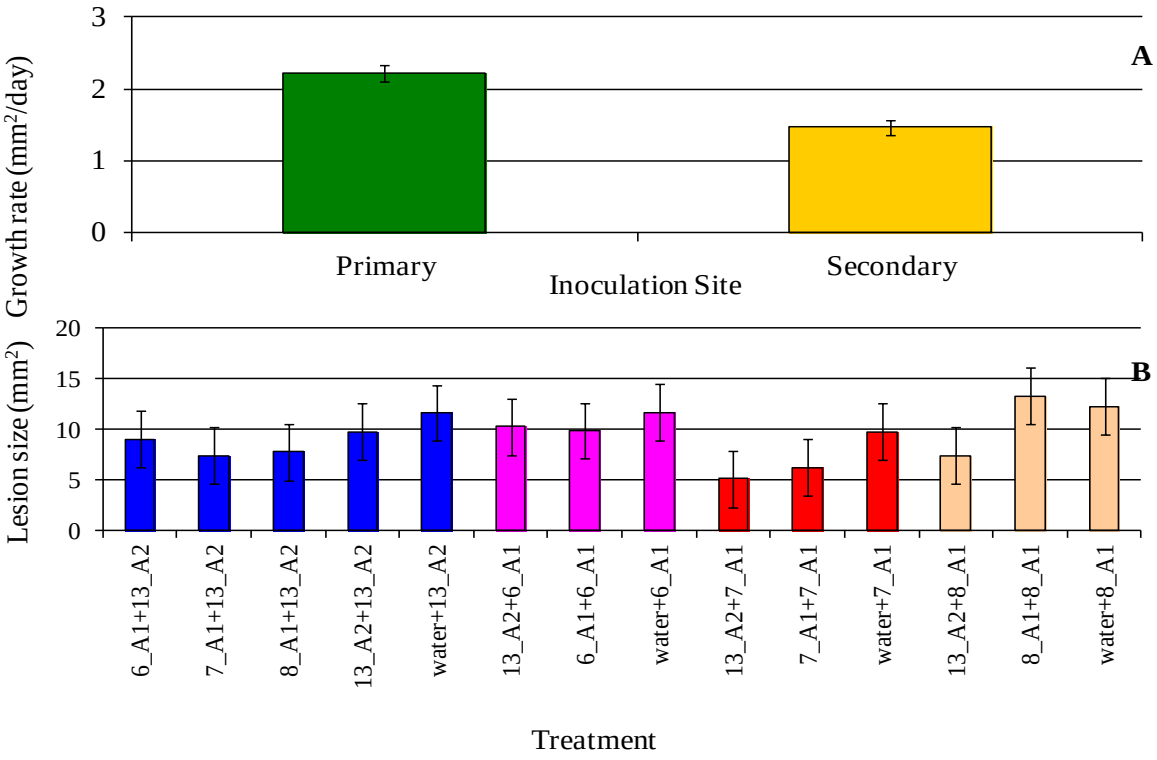


Figure 4.13 – Mean growth rate (mm^2/day)/lesion size (mm^2) for four UK *P. infestans* genotypes on whole detached potato leaflets representing the Near category. The error bars represent the standard error of the difference of the means. (SE).

A) Mean growth rate for each inoculation site. SE=0.11

B) Mean lesion size for each treatment at the secondary inoculation site (water control not shown or included in analysis). Colour of bars represent the genotype that was used as the secondary inoculation. SE=2.78

13_A2 as secondary inoculation

6_A1 as secondary inoculation

7_A1 as secondary inoculation

8_A1 as secondary inoculation

Table 4.10 – Descriptive statistics for lesion size values for the laboratory assay assessing the competitive ability of four UK *P. infestans* genotypes on whole detached leaflets at the Near inoculation site

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Inoculation Site	17	87.18	5.12	15.42	<.001
Treatment	13	221.44	17.03	1.47	0.196

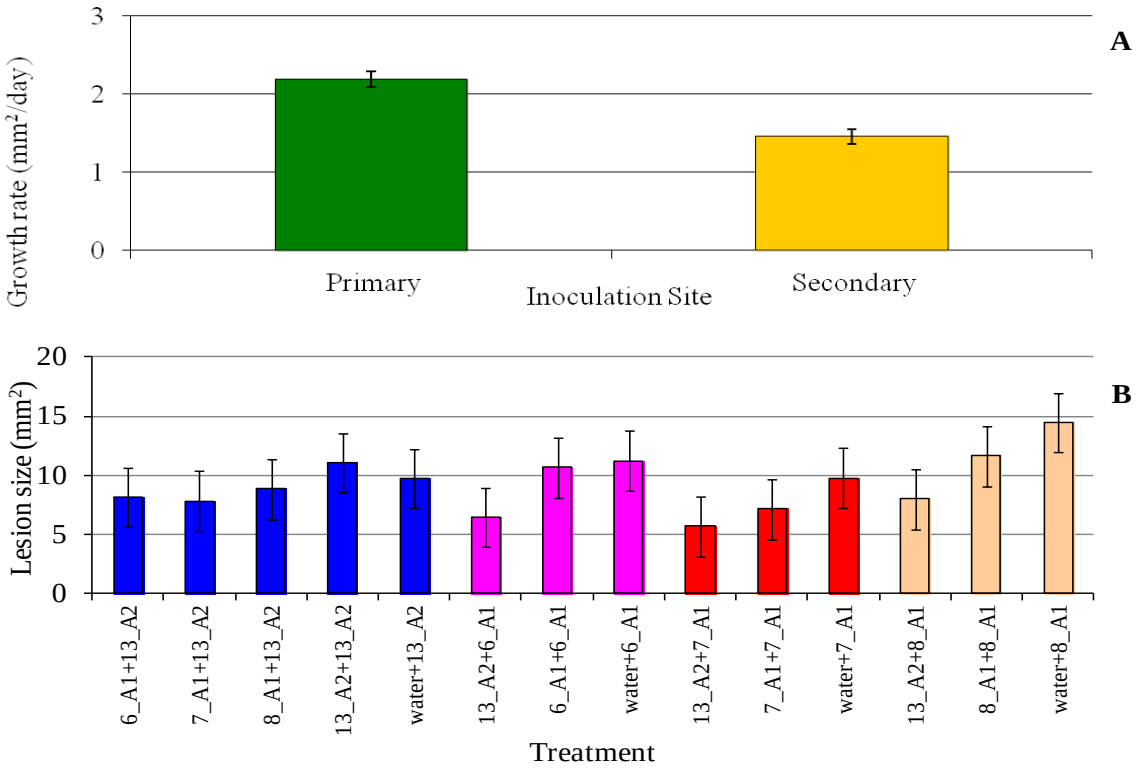


Figure 4.14 – Mean growth rate (mm²/day)/lesion size (mm²) for four UK *P. infestans* genotypes on whole detached potato leaflets representing the Far category. The error bars represent the standard error of the difference of the means (SE)

A) Mean growth rate for each inoculation site. SE=0.09

B) Mean lesion size for each treatment at the secondary inoculation site (water control not shown or included in analysis). Colour of bars represent the genotype that was used as the secondary inoculation. SE=2.78

13_A2 as secondary inoculation

6_A1 as secondary inoculation

7_A1 as secondary inoculation

8_A1 as secondary inoculation

Table 4.11 – Descriptive statistics for lesion size values for the laboratory assay assessing the competitive ability of four UK P. infestans genotypes on whole detached leaflets at the Far inoculation site

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio	P Value
Inoculation Site	17	54.29	4.95	20.45	<.001
Treatment	13	217.01	16.69	1.76	0.108

4.4 Discussion

4.4.1 Field trial

4.4.1.1 Spread of disease between plots

Spread of disease between plots was seen even though guard plants of potato cultivar Sárpo Mira, which has an extremely high level of resistance, were planted around each plot. In fact, only hypersensitive response (HR) symptoms were seen on the leaves of Sárpo Mira, and only when leaflets were detached from the plant did sporulation occur. Nonetheless, the foreign genotypes identified in the plots could have been spread by wind or when the plot was being scored as all plots were walked through in order to assess and sample each plant. Planting the plots further away from each other with barriers of another species could have aided in reducing the spread via the wind. To stop spread via walking through the plots the scorer could wear waterproof trousers and wash them off before examining each plot or disposable coveralls could be used. The difference between the proportion genotype 6_A1 in the 'all plots' pie charts (Figure 4.7.A) and the treatment pie chart (Figure 4.7.B) can be explained by the invading genotype 6_A1 samples that were found in plots of different treatments. Foreign samples invading plots were not taken into account when constructing pie charts so that a true genotype comparison within the treatments could be seen.

4.4.1.2 Genotyping

Out of the 994 samples collected, 158 samples could not be characterised into genotypes as a full complement of markers were not obtained due to poor initial template quality i.e. the sample having only small amounts of *P. infestans* DNA present. Some samples, especially from Cara, were hard to obtain as infection occurred on the lower leaflets of the plants which tended to be yellowing and senescing. This meant that, although the number of lesions may have been abundant enough for a

sample to be taken, the lesions were small and not sporulating as these leaves were less suitable for colonisation by *P. infestans*, compared to healthy leaves. Non-sporulating lesions had to be collected because it is important to find out which genotype was first to infect the plant. Samples that were not sporulating were incubated in a plastic bag for one night to promote sporulation and then the lesions were sampled onto an FTA card, but sporulation did not always occur. Those that did not sporulate after one night at high humidity could have been pressed in between two halves of a tuber in order promote sporulation, but the man power and time needed to do this would be great as there were so many samples and this could have led to bacterial growth on the sampled leaflet.

4.4.1.3 Genotype 13_A2

In both this field trial and in the Cooke *et al*. (2012a) field trial, genotype 13_A2 was the dominant genotype in terms of abundance. Genotype 6_A1 was the most aggressive genotype used in the plots in terms of the amount of foliar blight present on the inoculated plants (plant 1 and plant 16). Genotype 13_A2 spread faster within the plots compared to the other genotypes; dominance could therefore be due to how genotype 13_A2 spreads within the crop rather than how aggressive it is on a single plant. Genotype 13_A2 is more aggressive at lower temperatures which would allow it to infect sooner than the other genotypes and to grow better at night time when the temperatures are cooler. Rotem and Cohen (1974) looked at late blight epidemiology in semi-arid countries. They suggested that survivability was due to dispersal. Dispersal of sporangia is favoured by hot and dry conditions as it reached a peak rapidly and then stopped, whereas on a cool humid day, dispersal reached its peak more gradually and ebbed away. Dispersal seems to be very important in 13_A2 dominance. It could be that at the lower temperatures genotype 13_A2 sporulates and disperses its sporangia much sooner than the other genotypes and for a longer period of time thus giving it a

competitive advantage. It was assumed that the rates of 'escaping' samples were the same for genotype 6_A1 and 13_A2. If genotype 13_A2 was sporulating for longer it is unlikely that the rate of escaping samples was the same and most likely 13_A2 had a higher ESCAPE rate than 6_A1.

Genotype 13_A2 is resistant to the fungicide metalaxyl and evidence by Kadish and Cohen (1988) showed that isolates that were resistant to metalaxyl had a higher fitness index than isolates which were sensitive. The competitive fitness of *P. infestans* isolates from Israel that were either metalaxyl-resistant or metalaxyl-sensitive was tested on glasshouse grown potato plants of the cultivar Alpha. Infection frequency, lesion area and the sporulation capacity of 20 *P. infestans* isolates was recorded; ten isolates were resistant to metalaxyl and ten isolates were sensitive to metalaxyl. Epidemics in polytunnels were also assessed using potato cultivar Alpha with six isolates; three metalaxyl-resistant and three metalaxyl-sensitive isolates. Isolates that were metalaxyl-resistant had a larger lesion area than the isolates that were sensitive to metalaxyl but the infection frequencies and sporulation capacities were not significantly different. Epidemics in the polytunnels showed that metalaxyl-resistant isolates had a larger AUDPC value than the metalaxyl-sensitive isolates. Even in the absence of the selection pressure metalaxyl provides, those isolates that were resistant to it had a higher level of fitness than isolates that were metalaxyl-sensitive. Although, genotype 13_A2 had an advantage due to being resistant metalaxyl, this does not mean that there is a link between fitness and fungicide resistance. For example, Dowley and O'Sullivan (1985) found that when the application of metalaxyl is reduced there are fewer metalaxyl resistant isolates sampled in a field. If there was a genetic link between fitness and metalaxyl insensitivity, metalaxyl insensitivity would be just as prevalent in the years in which the fungicide is not used.

In this study, genotype 13_A2 did not produce the largest AUDPC value on single plants but it did have the most samples found within the plots, showing that the competitive fitness of genotype 13_A2 was higher than that of the other genotypes. Kadish *et al*. (1990) went on to look at how cultivar resistance affected the fitness of isolates that were metalaxyl-resistant and metalaxyl-sensitive. Three isolates of each metalaxyl-resistant and metalaxyl-sensitive population were used to infect 14 potato cultivars and fitness components were recorded; infection frequency, lesion area, sporulation capacity in leaflets and sporulation capacity in tuber disk. Metalaxyl-resistant isolates had larger lesions areas on susceptible cultivars than metalaxyl-sensitive isolates but there was no significant difference between the fitness levels of resistant and sensitive isolates on cultivars that were resistant. In this study, Cara (resistant) did have less disease present than Maris Piper (susceptible) but whether this was due to metalaxyl-resistant or sensitive genotypes was hard to determine as no differences were seen in the number of samples of each genotype for the cultivars.

4.4.1.4 Weather data

The Smith Period states that in order for there to be a risk of infection the minimum temperature must be 10°C or above with 90% RH for 11 or more hours for two consecutive days (Smith, 1956). Only two Smith Periods occurred during the epidemic and these occurred 19/20 days and 34/35 days post inoculation. Blight had already set in before the first Smith Period but between days 19 and 23 there was a large increase in the amount of blight present (showed in foliar blight present on the score days around that time). The fact that irrigation was used during the epidemic would have affected the RH within the canopy of the plots but would have not been identified by the weather station, which was not located within the crop. There would have been missed Smith Periods but this would be due to the limitations in the measurability of RH rather than limitations of the Smith Period.

4.4.2 Laboratory assay

4.4.2.1 Plant defences

The primary inoculation affected the secondary infection and this could be due to the plant defence responses triggered by the first infection. When the plant recognises infection by *P. infestans* a cascade of defence responses are triggered in particular the release of signalling molecules like salicylic acid (SA) that promotes the phenomenon of systemic acquired resistance. This starts the production of PR (pathogenesis-related) proteins and can lead to resistance to further attacks (Durrent and Dong, 2004).

There was no clear trend seen for the effect of the primary inoculation on the IP values of the secondary inoculation but trends were seen for the LP values. The difference was only significant for genotype 8_A1 and more pronounced when the secondary inoculation was on a different leaflet to the primary inoculation as opposed to the same leaflet as the primary inoculation. Genotype 6_A1, 7_A1 and 8_A1 all had longer LP values when the pre-inoculations were with the same genotype than when they were with water or 13_A2. However, for genotype 13_A2, the LP values were not affected by the primary inoculation. For lesion size, genotypes 6_A1, 7_A1 and 8_A1 had smaller lesions when the primary inoculation was genotype 13_A2 and the same genotype, although to a lesser degree for the same genotype, for both the near and far inoculations. There was a reduction in the size of the lesion for 13-A2 when the primary inoculation was a different genotype but it was less pronounced for genotype 13_A2 than the A1 genotypes. This showed that there was a consistent pattern for the genotypes in this test. However, it must be remembered that the genotypes in this study were only represented by one isolate so care must be taken in generalising these results to the entire genotype due to the amount of variation seen between isolates within genotypes (Chapter 3).

Clement *et al*. (2012) also examined the effect of a challenge inoculation with different or identical genotypes on the reproductive outcome of *P. infestans*. Five genotypes of *P. infestans*, all of the A1 mating type to rule out sexual reproduction and taken from two major potato production sites in France, were used in a detached leaflet assay. Inoculation occurred as either single-site infections (SSI) or double-site infections (DSI); a single leaflet was infected with one droplet for the SSI or two droplets (one each side of the midrib) for the (DSI). The DSI infections were either two isolates of the same genotype or one droplet of the genotype BP3 and one droplet of the other four genotypes; BEK, P13, P43 or PON05. Treatments affected the zoospore density of the isolates although there was no significant difference for SSI and DSI with two identical isolates. DSI always had an effect on the zoospore density but it differed for every genotype. Two strategies were observed; the genotypes BEK, P13 and PON05 produced more zoospores in the presence of different genotypes, while the genotypes BP3 and P4 produced more zoospores when other genotypes were absent. Clement *at al*. (2012) stated that the genotypes that have a higher reproductive fitness when infecting in the absence of another genotype will be at an advantage at the beginning of the epidemic but as the epidemic continues the genotypes that have a higher fitness in multiple infection situations will have the advantage. The study reported here contrasted with that of Clement *et al*., (2012) as all genotypes were affected when pre-inoculated with a *P. infestans* isolate. However, genotype 13_A2 was least affected by a pre-inoculation and this may give it an advantage over the other genotypes examined. This would mean that its dominance was due to factors other than aggressiveness (Chapter 3) and direct competitive ability. The present study focused on the competitive ability based on IP, LP and lesion size whereas Clement *et al*. (2012) used a genotype-specific qPCR protocol as a proxy for the quantification of zoospore density. It would have been interesting to extend the present study with qPCR

quantification of sporangial production to ascertain if this followed the same pattern as was seen for latent period and lesion size. Tooley and Fry (1985) compared the fitness of *P. infestans* isolates within a field trial using both lesion area population assessments and visual disease assessments and concluded that these methods gave different fitness estimates for the isolates although the isolate ranking orders were the same. Comparing latent period, lesion size and zoospore density could show different results due to the fact different components of fitness are being assessed.

4.4.3 Conclusion

In the field genotype 13_A2 proved to be dominant when compared to genotypes 6_A1, 7_A1 and 8_A1 on both Cara and Maris Piper, although when assessing the inoculated plants genotype 6_A1 was the most aggressive. Genotype 13_A2 therefore appears to gain its competitive advantage from its ability to spread from plant to plant rather than from its rate of colonisation of an infected plant. There was some evidence from the laboratory assay that the process of infection by genotype 13_A2 was less affected by the prior presence of infection by other genotypes or by itself. This may help to explain its competitive advantage in the field. Research on the interaction of temperature and humidity in the *P. infestans* infection process and their influence on sporulation are also needed as these may also contribute to giving genotype 13_A2 its competitive edge.

Chapter 5 – Effects of temperature on mycelial growth, infection, lesion expansion and sporulation of contemporary *P. infestans* genotypes

5.1 Introduction

During population change, genotypes that are displacing the current population are thought to have fitness benefits that give them a competitive advantage over their competitors. The nature of this fitness benefit may relate to novel biological traits that dictate the life cycle of the fitter genotypes. In 2005, the UK population of *P. infestans* underwent dramatic changes which led to genotype 13_A2 becoming dominant within the population from 2005 until 2010. There is evidence that similar occurrences have happened in other countries where newer, fitter genotypes displace the old population and become dominant. In the US, the US-1 genotype was displaced by newer genotypes, including US-7 and US-8. Mizubuti and Fry (1998) showed that the genotypes US-7 and US-8 both had lower optimal temperatures for sporangial germination than that of the 'old' genotype US-1. The sporangia of four isolates from the chosen genotype were transferred to water agar, and after 12 hours incubation at 10°C, 15°C, 20°C and 25°C the total number of sporangia that germinated was recorded. No significant differences were found between the genotypes at 10°C. Germination rates for all isolates decreased as the temperature increased, although at the higher temperatures genotype US-1 had the highest germination rate. The rate at which the sporangia germinated on water agar was assessed every two hours for one isolate from each genotype at 15°C. Variation between the genotypes was seen, for example, after two and four hours of incubation at 15°C US-8 and US-7 had significantly more germinated sporangia when compared to US-1. After six and eight hours of incubation at 15°C, there was no significant difference between the genotypes so although the number of sporangia germinating was the same after 6 hours, genotypes US-7 and US-8 germinated more rapidly than US-1. A detached leaflet assay was used to test the effect

of temperature upon fitness components of US-1 (three isolates), US-7 (four isolates) and US-8 (four isolates) at temperatures 10°C, 15°C, 20°C and 25°C. IP was the shortest for US-7 when compared to the other genotypes. US-8 had a significantly larger lesion area than US-1 and US-7. Genotypes US-7 and US-8 germinated sooner at lower temperatures when compared to US-1, favouring indirect germination. The release of zoospores leads to a larger number of infections, increasing the chance of establishing an infection and epidemic before the other genotypes. More rapid germination is likely to increase the likelihood of disease under marginal conditions giving that genotype an advantage over the other genotypes that germinate more slowly. This study also highlighted that US-7 had the shortest latent period of the genotypes tested and US-8 produced the largest lesion area. A short latent period means that US-7 completes its life cycle sooner than US-1; sporulating sooner than other genotypes will increase the chance of infecting the next host first thus increasing the rate of disease spread. The larger lesions produced by US-8 mean that this genotype would colonise the plant more rapidly and, in theory, spread through the crop more quickly as larger lesions provide a greater area for sporulation. Maziero et al., (2009) compared genotype US-1 and a Brazilian genotype called BR-1 and reported that US-1 produced more lesions on whole tomato plants at 15°C but the BR-1 isolate generated more lesions at 10°C. Recent studies by Cooke *et al* (2012a) have shown that genotype 13_A2 was more aggressive at 13°C compared to 18°C and suggested that genotype 13_A2 is better adapted to lower temperatures. Studies on low temperature survival provide other examples of differential responses to temperature in *P. infestans*. Survival rates at temperatures around freezing are an important factor for the presence of primary inoculum for the next season. Crosier (1934) found that sporangia could survive at -8°C for 16 hours and at -1.5°C for 27 hours and were still are able to infect host tissue. More recently, Kirk (2003) exposed isolates of a range of genotypes grown on agar to a

temperature of -3°C for 3-5 days and then incubated the isolates for 28 days at 12°C. The isolates that had been exposed to -3°C for up to three days could still grow when subsequently incubated at 12°C, but those exposed to -3°C for 4 and 5 days could not. Isolates of US-8 and US-14 could tolerate lower temperatures than isolates of US1. Survival at lower temperatures would aid in the overwintering process allowing more inoculum to be active in the next season.

5.1.1 Aim

The Smith Period criteria state that the minimum temperature required for there to be a potential blight risk is 10°C (Smith, 1956). The recent changes in the UK *P. infestans* populations have led to a need to re-evaluate the Smith Period criteria for the current population to provide more accurate biological data to validate the blight forecasting systems. The aim of this study was to assess the effects of temperature upon growth and sporulation of isolates of 11 UK *P. infestans* genotypes to examine whether differences in temperature response may contribute to the fitness advantage of new lineages. Such data will also be an important consideration in predicting periods of pathogen activity required for accurate decision support systems. The studies were conducted with a combination of growth rate experiments on Rye A agar, in which colony size was measured, and aggressiveness studies on detached leaflets of the potato cultivar Maris Piper, in which the fitness components IP, LP and LS were measured.

5.2 Methods

5.2.1 *In vitro* growth

An *in vitro* study of 52- 56 *P. infestans* isolates, comprising 11 genotypes, was conducted on Rye A agar plates (Rye A ingredients found in Chapter 2.1.2) to assess the effect of temperature on colony growth at six different temperatures; 5°C, 10°C, 15°C, 20°C, 25°C and 30°C.

5.2.1.1 Design

A fully randomised block design was used; isolates were randomised within four replicate blocks for each temperature. Before the test, all isolates were sub-cultured from archived slopes and grown on Rye A agar to regain normal growth of the isolates.

5.2.1.2 Plating and incubation

All isolates were subcultured from cultures already growing on Rye A agar (Table 5.1). Using a 5 mm steel corer that had been dipped in ethanol, flamed and allowed to cool, a small section of the mycelium from the edge of the growing colony was transferred to a fresh 9 cm Petri dish containing a set depth of Rye A agar. Four replicates plates were made for each isolate and all plates were sealed with Nescofilm. Each temperature experiment was run independently on the following dates; 5°C in November 2009, 10°C in March 2010, 15°C in May 2010, 20°C in June 2010, 25°C in November 2010 and 30°C in January 2011. All tests had a light/dark cycle of 16 hours light and 8 hours dark in an incubator (LMS cooled incubator).

5.2.1.3 Data collection and analysis

The growth of each isolate was scored at regular intervals using digital callipers (0-6”/150 mm digimatic calipers, Mitutoyo UK Ltd) by measuring the diameter of the colony in 2 directions; the width and length at 90° to each other. A final measurement

was taken when the first isolate reached the edge of the plate and the mean colony area was calculated. An analysis of variance (ANOVA) was conducted to examine the effect of temperature on the growth response of isolates and genotypes as discussed in Chapter 2.8.

5.2.2 *In vivo* growth

A detached leaflet assay using potato cultivar Maris Piper was used to test the growth of 47 *P. infestans* isolates on a temperature gradient plate (GRD1, Grant Instruments, Cambridge). All isolates used were passaged through the potato cultivar Craig's Royal (susceptible) twice before being used to make inoculum.

5.2.2.1 Design

Sixty four Petri dishes (9 cm) were placed on the temperature gradient plate in 8 columns of 8 Petri dishes lid side down (Figure 5.1.A, Table 5.2). Damp filter paper was used to line each lid and two detached Maris Piper leaflets were placed abaxial side up on the filter paper. Due to the limited size of the incubator, a series of experiments were run, in each of which 7 test isolates and one standard (reference) isolate was tested. Leaflets were randomised within the columns and the two replicates of each isolate were never paired together in the same Petri dish. Each column was randomised. The temperature gradient plate was set at 6°C to 20°C which generated a gradient across the plate with each column having a different temperature. Temperature loggers (DS1921G Thermochron iButton, Maxim) were placed along a central horizontal line in between the Petri dishes to record the temperature. The light and dark cycle of 16 and 8 hours respectively was programmed into the temperature gradient plate illuminated lid.

5.2.2.2 Inoculation

Seven day old sporulating lesions on detached Craig's Royal leaflets were used to make the sporangial suspension for each isolate as described in Chapter 2.4. The leaflets were

inoculated with a 15 µl droplet of sporangial suspension near the midrib of the leaflet on the abaxial surface, in the same place on each leaflet. The bases of the Petri dishes were placed on the lids to create a humid environment and extra water was added to the filter paper when needed in order to maintain the high humidity. The Petri dishes were shaded with blue tissue paper for the first 24 hours post-inoculation.

5.2.2.3 Data collection and analysis

Aggressiveness criteria were recorded; IP, LP and final LS. IP and LP were recorded when symptoms and sporulation were first visible to the naked eye and the final lesion size was measured with digital calipers (0-6"/150 mm digimatic calipers, Mitutoyo UK Ltd) at 2 orientations at right angles to each other. Mean lesion size was calculated and an ANOVA was run according to the statistical methods discussed in Chapter 2.8. Each genotype was represented by different numbers of isolates in each test. This was accounted for in the general ANOVA to provide the standard error for every genotype combination.

5.2.3 *In vivo* growth with diurnal temperature regimes

Four genotypes were tested using detached leaflets of the cultivar Maris Piper on the temperature gradient plate set to 6°C to 20°C with the gradient switching through 90° in each 24 hour period to generate different diurnal temperature regimes. Due to the constraints of space on the gradient incubator table, one isolate was used to represent each genotype; 13_A2 (2006_3928A), 6_A1 (2008_6090A), 2_A1 (2007_5442F) and 17_A2 (2006_4338D) (Table 5.2). All isolates were passaged through potato cultivar Craig's Royal (susceptible) twice before being used for inoculum.

5.2.3.1 Design

Forty nine square Petri dish (10.5 cm diameter) lids were lined with damp filter paper and placed in 7 columns of 7 Petri dishes (Figure 5.1.B). Four detached leaflet of potato

cultivar Maris Piper were placed in each corner of the Petri dishes making sure to not overlap them, in the centre a temperature logger (DS1921G Thermochron iButton, Maxim) was placed to record the temperature at each Petri dish. The temperature gradient plate was set from 6°C to 20°C and as the light and dark cycle switched over so did the direction of the gradient. For example in the 16 hour light period the gradient went from 6°C to 20°C from left to right, but in the 8 hour dark period the 6°C to 20°C gradient went from bottom to the top of the plate. Each Petri dish on the gradient plate was thus exposed to a different combination of temperatures. The experiment was repeated four times, each time rotating the genotype placement within the dish. This was to ensure that data for each isolate was recorded at all four corners to compensate for the small gradients in temperature across the dishes.

5.2.3.2 Inoculation

Sporangia were taken from sporulating lesions on potato cultivar Craig's Royal leaflets and inoculum was made as described in Chapter 2.4. Leaflets were inoculated with a 15 µl droplet of sporangial suspension near the midrib of the leaflet on the abaxial surface; all leaflets were inoculated in the same place. Bases of the Petri dishes were replaced to maintain humidity; water was added when needed to maintain humidity. The Petri dishes were shaded with blue tissue paper for the first 24 hours post-inoculation.

5.2.3.3 Data collection and analysis

Aggressiveness criteria was measured; IP, LP and final LS. IP and LP were scored based on when symptoms and sporulation were first viewed with the naked eye. Lesion size was scored with digital calipers as described above. Accumulated day degrees were calculated by multiplying the average day temperature by the number of hours in the day (16) and adding that to the average night temperature multiplied by the number of night hours (8). Statistical analysis was as described in Chapter 2.8.

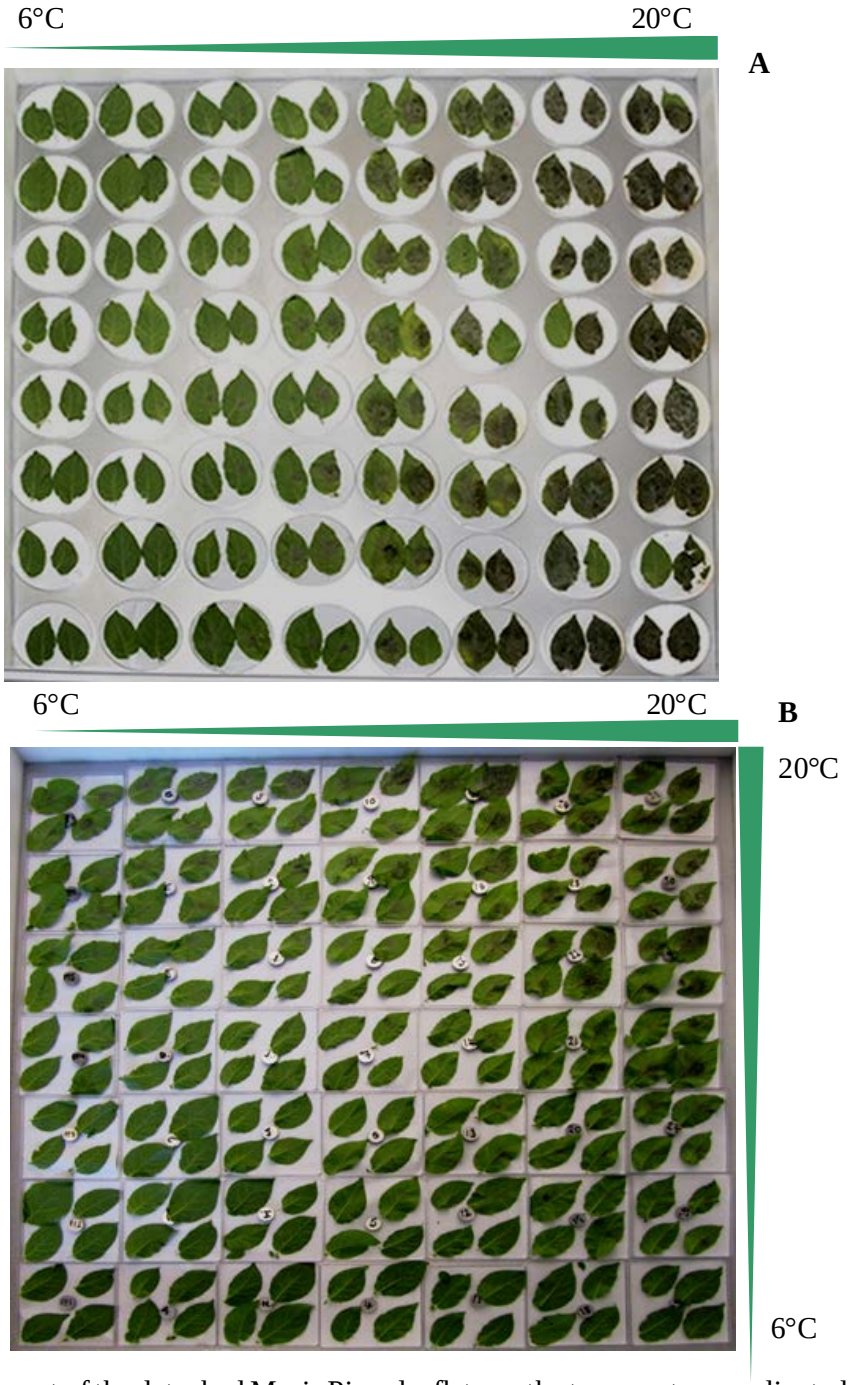


Figure 5.1 – Layout of the detached Maris Piper leaflets on the temperature gradient plate for A) the *in vivo* study and B) the *in vivo* with diurnal combinations study

Table 5.1 – Isolates used in the *in vitro* test at each temperature

Temperature	Genotypes/Isolates used at each temperature	No. of Isolates
5°C	1_A1 - 2006_3984C, 2007_5138G	2
	2_A1 - 2006_3888A, 2007_5442F, 2008_6850D	4
	3_A2 - 2006_4012F, 2007_5074E	2
	6_A1 - 2006_4100A, 2008_6090A, 2008_6306A, 2008_6354C, 2008_6426A, 2008_6498A, 2008_6502A, 2008_6610E, 2008_7034E	9
	7_A1 - 2006_4168B, 2007_5290C, 2007_5918A, 2006_6066A, 2008_6274D	5
	8_A1 - 2006_4232E, 2008_6070E, 2008_6222A, 2008_6422F, 2008_6458E	5
	10_A2 - 2006_4440C, 2007_5482D, 2007_5482E, 2007_5706E	4
	13_A2 - 07_39, 2006_3928A, 2008_6050B, 2008_6082F, 2008_6102A, 2008_6194A, 2008_6250A, 2008_6430A, 2008_6530C, 2008_7038A	10
	17_A2 - 2006_4388C, 2006_4388D	2
	A1 Misc – 88069, 2006_3996A, 2007_5054A, 2007_5738E, 2007_5738G, 2007_5974A, 2008_6394B, 2008_6446F, T30-4	9
	A2 Misc – 2007_5726C, 2008_6446D, Bayer 9B	4
10°C	1_A1 - 2006_3984C, 2007_5138G	2
	2_A1 - 2006_3888A, 2007_5442F, 2008_6850D	3
	3_A2 - 2006_4012F, 2007_5074E	2
	6_A1 - 2006_4100A, 2008_6090A, 2008_6306A, 2008_6354C, 2008_6426A, 2008_6498A, 2008_6502A, 2008_7034E	8
	7_A1 - 2006_4168B, 2007_5290C, 2007_5918A, 2006_6066A, 2008_6274D	5
	8_A1 - 2006_4232E, 2008_6070E, 2008_6222A, 2008_6422F, 2008_6458E	5
	10_A2 - 2006_4440C, 2007_5482D, 2007_5482E, 2007_5706E	4
	13_A2 - 07_39, 2006_3928A, 2008_6050B, 2008_6082F, 2008_6102A, 2008_6194A, 2008_6250A, 2008_6430A, 2008_7038A	10
	17_A2 - 2006_4388C, 2006_4388D	2
	A1 Misc – 88069, 2006_3996A, 2007_5054A, 2007_5738E, 2007_5738G, 2007_5974A, 2008_6394B, 2008_6446F, T30-4	9
	A2 Misc – 2007_5726C, 2008_6446D, Bayer 9B	3

Table 5.1 continued - Isolates used in the *in vitro* test at each temperature

Temperature	Genotypes/Isolates used at each temperature	No. of Isolates
15°C	1_A1 - 2006_3984C	1
	2_A1 - 2007_5442F, 2007_5622A, 2008_6850D, 2008_7006D	4
	3_A2 - 2006_4012F, 2007_5074E	2
	6_A1 - 2006_4100A, 2008_6090A, 2008_6306A, 2008_6354C, 2008_6426A, 2008_6498A, 2008_6502A,2008_6610E, 2008_7034E	9
	7_A1 - 2006_4168B, 2007_5290C, 2007_5918A, 2006_6066A, 2008_6274D	5
	8_A1 - 2006_4232E, 2008_6070E, 2008_6222A, 2008_6422F, 2008_6458E	5
	10_A2 - 2007_5482D, 2007_5706E	2
	13_A2 - 07_39, 2006_3928A, 2008_6050B, 2008_6082F, 2008_6102A, 2008_6194A, 2008_6250A, 2008_6430A, 2008_6530C, 2008_7038A	10
	17_A2 - 2006_4388C, 2006_4388D	2
	A1 Misc – 88069, 2006_3996A, 2007_5054A, 2007_5738E, 2007_5738G, 2007_5974A, 2008_6394B, 2008_6446F, T30-4	8
	A2 Misc – 2007_5726C, 2007_5738B, 2008_6446D, Bayer 9B	4
20°C	1_A1 - 2006_3984C, 2007_5138G	2
	2_A1 - 2006_3888A, 2007_5442F, 200+_6250A, 2008_6850D, 2008_7006D	5
	3_A2 - 2006_4012F, 2007_5074E	2
	6_A1 - 2006_4100A, 2008_6090A, 2008_6306A, 2008_6354C, 2008_6426A, 2008_6498A, 2008_6502A, 2008_6610E, 2008_7034E	9
	7_A1 - 2006_4168B, 2007_5290C, 2007_5918A, 2006_6066A, 2008_6274D	5
	8_A1 - 2006_4232E, 2008_6070E, 2008_6222A, 2008_6422F, 2008_6458E	5
	10_A2 - 2006_4440C, 2007_5482D, 2007_5706E	3
	13_A2 - 07_39, 2008_6050B, 2008_6102A, 2008_6194A, 2008_6250A, 2008_6430A, 2008_6530C, 2008_7038A	8
	17_A2 - 2006_4388C, 2006_4388D	2
	A1 Misc – 88069, 2006_3996A, 2007_5054A, 2007_5738E, 2007_5974A, 2008_6394B, 2008_6446F, T30-4	8
	A2 Misc – 2007_5726C, 2007_5738B, 2008_6446D, Bayer 9B	4

Table 5.1 continued - Isolates used in the *in vitro* test at each temperature

Temperature	Genotypes/Isolates used at each temperature	No. of Isolates
25°C	1_A1 - 2006_3984C, 2007_5138G	2
	2_A1 - 2006_3888A, 2007_5442F, 2008_5622A, 2008_6850D, 2008_7006D	5
	3_A2 - 2006_4012F, 2007_5074E	2
	6_A1 - 2006_4100A, 2008_6090A, 2008_6306A, 2008_6354C, 2008_6426A, 2008_6498A, 2008_6502A, 2008_6610E, 2008_7034E	9
	7_A1 - 2006_4168B, 2007_5290C, 2007_5918A, 2006_6066A, 2008_6274D	5
	8_A1 - 2006_4232E, 2008_6070E, 2008_6222A, 2008_6422F, 2008_6458E	5
	10_A2 - 2006_4440C, 2007_5482D, 2007_5482E, 2007_5706E	4
	13_A2 - 2006_3928A, 2008_6050B, 2008_6082F, 2008_6102A, 2008_6194A, 2008_6250A, 2008_6430A, 2008_7038A	9
	17_A2 - 2006_4388C, 2006_4388D	2
	A1 Misc – 88069, 2006_3996A, 2007_5054A, 2007_5738G, 2007_5974A, 2008_6394B, 2008_6446F, T30-4	8
	A2 Misc – 2007_5726C, 2008_6446D, Bayer 9B	3

Table 5.2 - Isolates used in the *in vivo* tests

Experiment	Genotypes/Isolates used at each temperature	No. of Isolates
In vivo	1_A1 - 2006_3984C, 2007_5138G	2
	2_A1 – 2006_3888A, 2008_6850D, 2008_7006D	3
	3_A2 – 2006_4012F, 2007_5074E	2
	6_A1 – 2008_6090A, 2008_6306A, 2008_6354C, 2008_6426A, 2008_6498A, 2008_6502A, 2008_6610E, 2008_7034E	8
	7_A1 – 2006_4168B, 2007_5290C, 2007_5918A, 2008_6274D	4
	8_A1 – 2006_4232E, 2008_6222A, 2008_6422F, 2008_6458E	4
	10_A2 – 2006_4440C, 2007_5482D, 2007_5482E	3
	13_A2 – 07_39, 2006_3928A, 2008_6050B, 2008_6082F, 2008_6102A, 2008_6194A, 2008_6250D, 2008_6430A, 2008_6530C, 2008_7038A	10
	17_A2 – 2006_4388C, 2006_4388D	2
	A1 Misc – 88069,2007_5054A, 2007_5738G, 2007_5974A, 2008_6070E, 2008_6394B, 2008_6446F	7
	A2 Misc – 2007_5726C, Bayer 9B	2
Diurnal *in vivo*	2_A1 – 2007_5442F	1
	6_A1 – 2008_6090A	1
	13_A2 – 2006_3928A	1
	17_A2 – 2006_4388D	1

5.3 Results

5.3.1 *In vitro* growth

5.3.1.1 Isolate

Since the isolates grew at different rates for each temperature, the final measurements were taken at 43, 20, 15, 9, 7 and 21 days after the colonies were established for the temperatures 5, 10, 15, 20, 25 and 30°C respectively. No growth was recorded in any of the isolates at 30°C so this temperature was excluded from the analysis. The mean final colony size of isolates at each temperature was compared (Appendix iii). To make comparisons between temperatures, the colony growth rates (mm per day) were calculated from the final colony size divided by the number of days of incubation. Colony growth rate ranged from a maximum of 12.3 mm per day in an isolate at 25°C to a minimum rate of 0.2 mm per day at 5°C. The majority of isolates grew most rapidly at 20°C (Fig. 5.2). There were statistically significant differences in final colony size and growth rate of the tested isolates at all temperatures (P=0.001, Table 5.3, Appendix iii). The pattern was, however, complex with no single isolate showing the most consistently rapid or slow growth across the whole range of temperatures. The replicate isolates representing each pathogen genotype did not necessarily grow at similar rates and, in several cases, statistically significant differences in responses to temperature were observed within these groups.

Isolate 2007_5074A (genotype 3_A2) had the largest colony size at temperatures 5°C (58.76 mm^2), 10°C (78.15 mm^2), 15°C (82.28 mm^2) and at 20°C (81.78 mm^2). At 25°C the isolate 2006_6446D (genotype A2 Misc) had the largest colony size with 84.17 mm^2. The isolate with the smallest colony size was different at most temperatures; isolate 2007_5738B (genotype A2 Misc) with 0.57 mm^2 at 5°C, isolate 2008_6222A (genotype 8_A1) with 20.32 mm^2 at 10°C, isolate 2007_5974A (genotype A1 Misc) at

15°C (24.83 mm^2) and at 20°C (18.89 mm^2) and isolate 2008_6274D (genotype 7_A1) with 1.96 mm^2 at 25°C. Rather than describe, in detail, the growth of all isolates, the section below focuses on the largest groups of isolates from genotypes 13_A2 and 6_A1.

At 5°C, isolates representing genotype 13_A2 had a wide range of colony sizes. A group of three isolates had amongst the largest colonies of all isolates at this temperature (2008_6194A, 2008_6530C and 2008_7038A), four showed intermediate growth (2008_6082F, 2006_6250A, 2008_6050B and 2008_6102A) whereas two grew poorly (2008_6430A and 2006_3928A). The nine isolates representing genotype 6_A1 indicated a similar range of growth as 13_A2 with two isolates being significantly larger than the others (2008_6306A and 2008_6345C), four with an intermediate colony size (2008_6090A, 2008_6498A, 2008_7034A and 2008_6502A) and two isolates with colonies significantly smaller than all other 6_A1 isolates (2008_6426A and 2006_4100A). In general, isolates of the genotypes 13_A2 had larger colonies than 6_A1 at 5°C.

Less variation between all isolates was seen at 10°C. Isolates of genotype 13_A2 had intermediate to small colony sizes compared to incubation at 5°C. The 13_A2 isolate that grew best at 5°C (2008_6530C) also had colonies significantly larger than all other 13_A2 isolates except 2008_6194A. As with its growth at 5°C, isolate 2008_6430A had the smallest colony at 10°C. Isolates representing genotype 6_A1 had a wide range of colony sizes; the colonies of four isolates (2008_6090A, 2008_6502A, 2008_7034E and 2006_4100A) were significantly larger than the other 6_A1 isolates. Isolate 2008_6498A that grew least at 10°C did not have the smallest colony at 5°C.

At 15°C, the colony sizes were more uniform across the tested isolates, for example there were no statistically significant differences amongst the 20 isolates with the largest

colonies. Isolates of the genotype 13_A2 showed little variation and most had large to intermediate colonies. Of the 10 isolates that represented genotype 13_A2 only two (2008_6082F and 2008_6530C) had significantly smaller colonies than the rest. Interestingly, one of these isolates (2008_6530C) had the largest colonies of all the 13_A2 isolates at 5 and 10°C. It was also of note that amongst isolates of genotype 6_A1 only three (2008_6502A, 2008_6090A and 2008_6050B) were within the top 20 isolates with the largest colonies. The other 6_A1 isolates had amongst the smallest colonies . Isolate 2006_4100A which had grown well at 10°C had, along with isolate 2008_7034E, significantly smaller colonies than all other 6_A1 isolates at 15°C.

At 20°C, a wide range of colony sizes were observed amongst isolates that represented genotype 13_A2. Statistically significant differences were noted between the pairs of isolates with the largest, intermediate and smallest colonies. Isolate 2008_6350C with the smallest 13_A2 colony at 20°C formed the second largest and largest of the 13_A2 isolate colonies at 5 and 10°C respectively. A similar pattern was observed amongst genotype 6_A1 with isolates forming some of the largest and smallest colonies at this temperature.

With the exception of isolate 2008_6446D (misc A2) and 2006_3928A (13_A2), most isolates grew less well at 25°C. Apart from isolate 2006_3928A, 13_A2 isolates grew poorly at 25°C. Isolates representing genotype 6_A1 grouped within the mid to lower range of growth but on average, grew better than isolates of 13_A2 at this temperature.

5.3.1.2 Genotype

When the individual isolate data was grouped into the 11 genotype categories, the statistical analysis indicated a significant variation in colony size according to genotype at each temperature ($P<.001$, Figure 5.2, Table 5.4). The mean growth rate of all isolates at each temperature differed significantly from other temperatures with the growth rate at 20°C being the largest and also, on average, the optimum temperature for colony growth of all genotypes (Figure 5.2.A). The mean growth rates at 10°C and 15°C were greater than that at 25°C. Although some individual isolates failed to grow at 5°C, some growth was observed in each of the 11 genotype groups. No growth was seen at 30°C for any genotype. The growth rates broadly follow the same pattern for each genotype, i.e. a low growth rate at 5°C, which gradually increases as the temperature rises to 20°C, then growth was reduced significantly as the temperature reached 25°C and 30°C (Figure 5.2.B-G).

At each of the temperatures tested there were statistically significant differences in mean colony size amongst the genotypes ($P <.001$, Figure 5.3, Table 5.4). Strikingly, genotype 3_A2 had significantly larger colonies compared to the other genotypes at all temperatures except 25°C. Genotype 13_A2 produced colonies that were of an intermediate size at each temperature and were consistently ranked third or fourth in colony size out of 11 genotypes with the exception of temperature 25°C where it dropped to seventh. Genotype 3_A2, 17_A2 and 13_A2 were consistently ranked within the top six genotypes with the largest colony sizes. However, not all genotypes were consistent in the ranking orders for each temperature. For example, the mean growth rate of the 6_A1 isolates was in the middle to low range at most temperatures but the second highest at 10°C (Figure 5.2). The same inconsistency in the ranking orders for genotypes 2_A1, 8_A1 and 10_A2 was also observed (Figure 5.2).

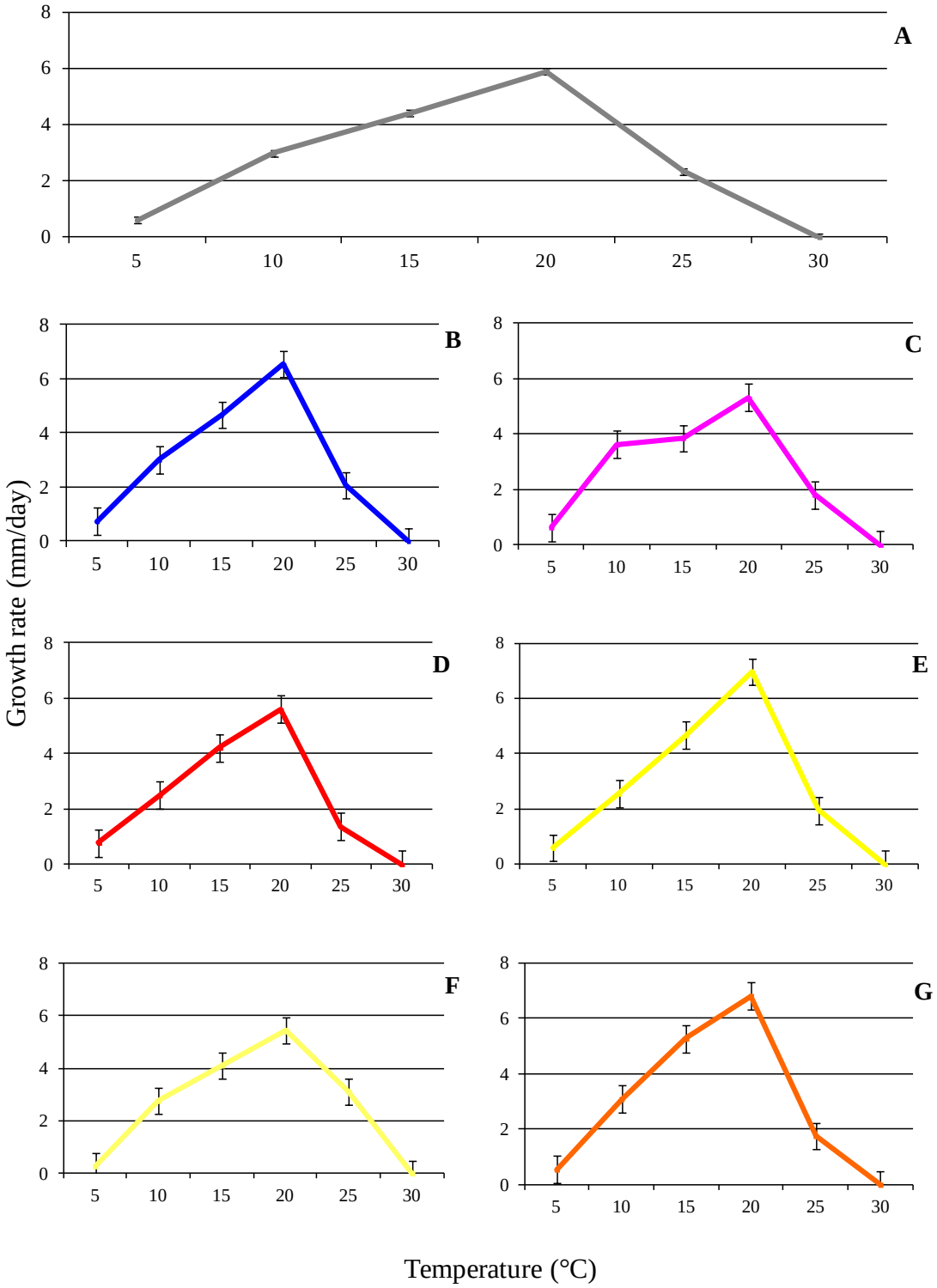


Figure 5.2 – Mean growth rate (mm/day) for UK *P. infestans* genotypes at a range of temperatures from 5°C-30°C. The error bars represent the standard errors of the differences of the mean (SE). SE for all genotypes is 0.12, SE for individual genotypes is 0.49.

A) All genotypes B) Genotype 13_A2 C) Genotype 6_A1 D) Genotype 8_A1
E) Genotype 10_A1 F) Genotype A2 Misc G) Genotype 2_A1

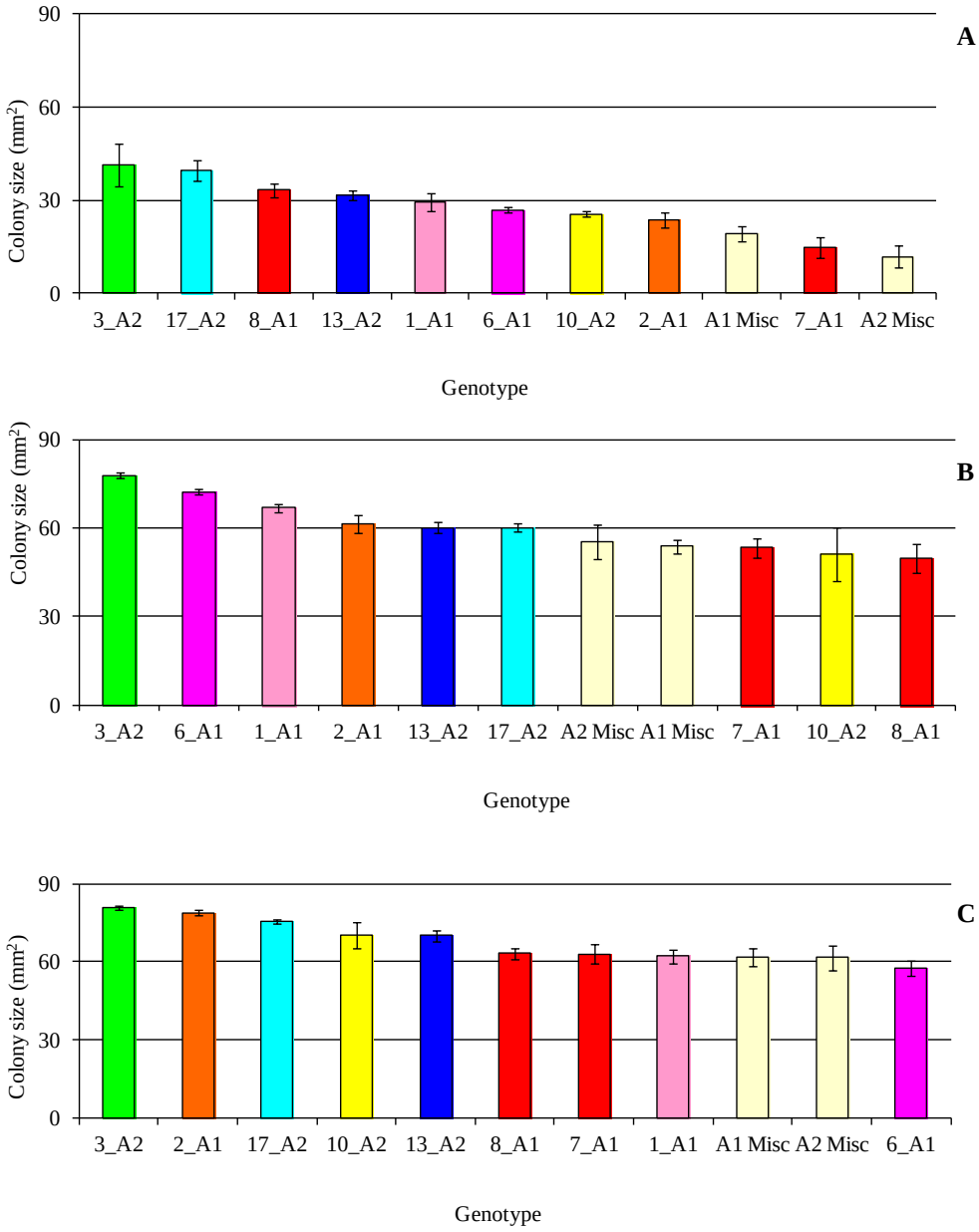


Figure 5.3 – Mean colony size (mm^2) for UK *P. infestans* genotypes grown on Rye A agar at a range of temperatures. The error bars represent the standard errors of the differences of the mean (SE). Number of isolate representing each genotype is stated after SE

A) 5°C SE= 3_A2 6.72 (n=2), 17_A2 3.23 (n=2), 8_A1 2.29 (n=5), 13_A2 1.54 (n=10), 1_A1 2.93 (n=2), 6_A1 0.94 (n=9), 10 A2 1.05 (n=4), 2_A1 2.28 (n=4), A1 Misc 2.46 (n=9), 7_A1 3.25 (n=5) and A2 Misc 3.62 (n=4)

B) 10°C SE= 3_A2 0.81 (n=2), 6_A1 0.81 (n=8), 1 A1 1.22 (n=2), 2 A1 3.02 (n=3), 13_A2 1.26 (n=10), 17_A2 1.27 (n=2), A2 Misc 5.64 (n=3), A1 Misc 2.16 (n=9), 7_A1 3.14 (n=5), 10 A2 9.03 (n=4) and 8_A1 4.84 (n=5)

C) 15°C SE= 3_A2 0.57 (n=2), 2_A1 0.96 (n=4), 17_A2 0.94 (n=2), 10 A2 5.27 (n=2), 13_A2 2.09 (n=10), 8_A1 2.10 (n=5), 7_A1 3.73 (n=5), 1_A1 2.74 (n=1), A1 Misc 3.28 (n=8), A2 Misc 4.64 (n=4) and 6_A1 3.06 (n=9)

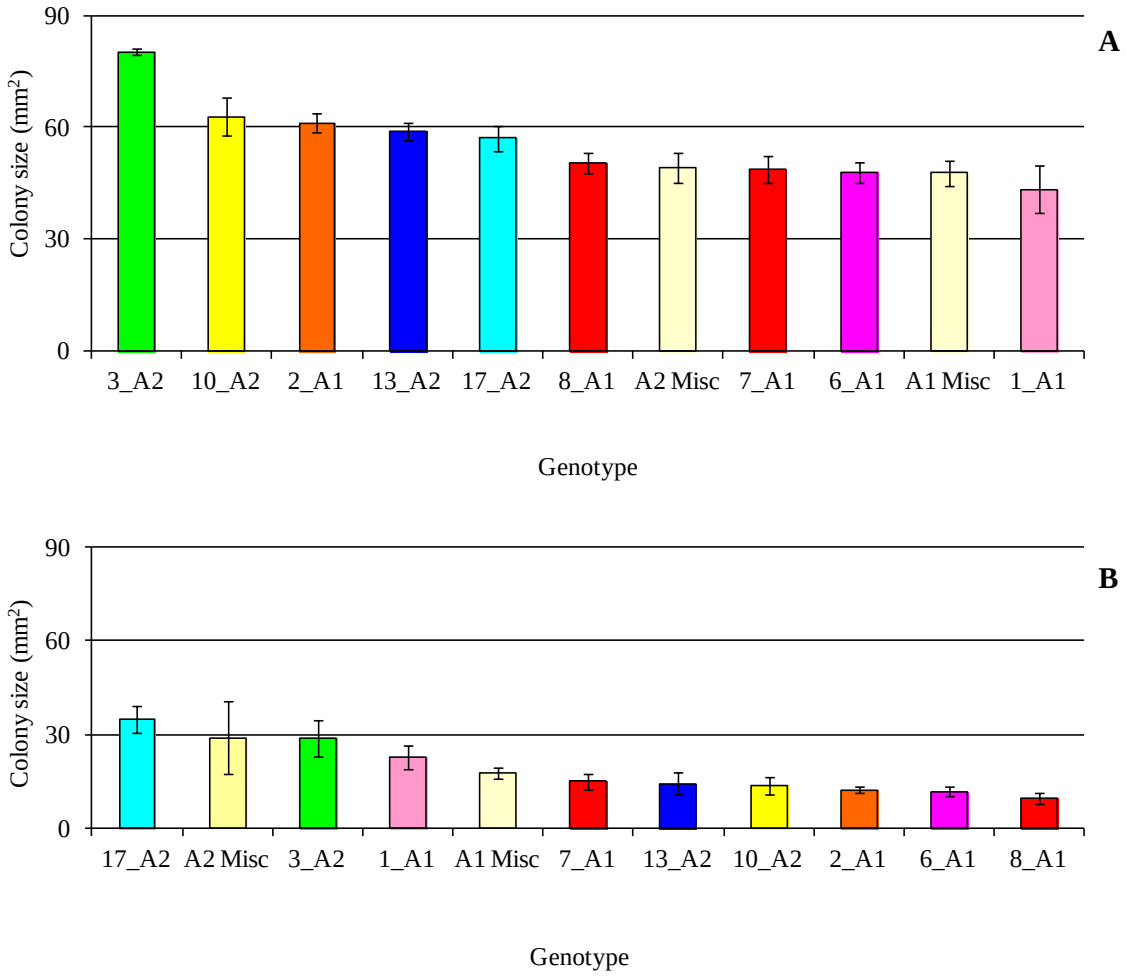


Figure 5.3 continued – Mean colony size (mm^2) for UK *P. infestans* genotypes grown on Rye A agar at a range of temperatures. The error bars represent the standard errors of the differences of the mean (SE). Number of isolate representing each genotype is stated after SE

A) 20°C SE= 3_A2 0.90 (n=2), 10_A2 5.11 (n=3), 2_A1 2.53 (n=5), 13_A2 2.42 (n=8), 17_A2 3.36 (n=2), 8_A1 2.66 (n=5), A2 Misc 3.92 (n=4), 7_A1 3.60 (n=5), 6_A1 2.72 (n=9), A1 Misc 3.55 (n=8) and 1_A1 6.29 (n=2)

B) 25°C SE= 17_A2 4.26 (n=2), A2 Misc 11.79 (n=3), 3_A2 5.74 (n=2), 1 A1 3.65 (n=2), A1 Misc 1.72 (n=8), 7_A1 2.35 (n=5), 13_A2 3.51 (n=9), 10_A2 2.62 (n=4), 2_A1 1.09 (n=5), 6_A1 1.44 (n=9) and 8_A1 1.76 (n=5)

Table 5.3 – Descriptive statistics for *in vitro* temperature investigations; testing how temperature affects colony size of *P. infestans* isolates on Rye A agar plates. Variation in the degrees of freedom relate to isolates recorded as 'missing data' due to factors such as bacterial or fungal contamination.

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
5°C	Colony size	55	34922.93	634.96	30.84	<.001
10°C	Colony size	52	55504.2	1067.39	62.6	<.001
15°C	Colony size	51	49046.16	961.69	39.63	<.001
20°C	Colony size	52	55081.53	1059.26	46.85	<.001
25°C	Colony size	53	52961.02	999.26	57.17	<.001

Table 5.4 – Descriptive statistics for *in vitro* temperature investigations; testing how temperature affects colony size of *P. infestans* genotypes on Rye A agar plates.

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
All temps	Colony size (mm/day)	5	5729.10	1145.82	640.44	<.001
5°C	Colony size (mm^2)	10	13316.30	1331.60	11.29	<.001
10°C	Colony size (mm^2)	10	14251.70	1425.20	6.39	<.001
15°C	Colony size (mm^2)	10	10708.60	1070.90	5.02	<.001
20°C	Colony size (mm^2)	10	13738.80	1373.90	5.79	<.001
25°C	Colony size (mm^2)	10	9239.00	923.90	3.85	<.001

5.3.2 *In vivo* growth study

5.3.2.1 Temperature regulation

The output from the data loggers indicated only minor variation (+/- 0.5°C) in the temperature over each 24 hour time period as the heat from the lights increased the temperature slightly (Figure 5.4.A). The recorded temperature was consistent with that to which the machine was set with minor differences due to the fact that the iButtons were not placed exactly in line with the columns of the Petri dishes but in the spaces between the columns. Although the temperature gradient plate was set from 6°C to 20°C, at the coolest point on the plate the temperature reached 5°C.

5.3.2.2 Reference isolates

The standard reference isolate used in each separate test was 2006_3928A (genotype 13_A2). No significant differences in IP and lesion size values for the reference isolate were seen between tests for each temperature except for temperatures 14°C and 16°C for IP and 20°C for lesion expansion (Table 5.5 and Table 5.6). The data for tests and temperatures at which there was no significant difference in the performance of the standard isolate were combined and thus analysed as a whole data set rather than as separate tests.

Table 5.5 – Descriptive statistics for variation between the IP values of the standard isolate used on the *in vivo* temperature investigations.

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
6°C	Experiment	7	0.938	0.134	0.430	0.859
8°C	Experiment	7	7.438	1.062	0.89	0.552
10°C	Experiment	7	9.438	1.348	2.400	0.122
12°C	Experiment	7	21.438	3.062	1.960	0.183
14°C	Experiment	7	6.938	0.991	5.290	0.016
16°C	Experiment	7	11.000	1.571	4.190	0.031
18°C	Experiment	7	8.938	1.277	1.080	0.455
20°C	Experiment	7	5.000	0.714	2.860	0.082

Table 5.6 – Descriptive statistics for variation between the LS values of the standard isolate used on the *in vivo* temperature investigations

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
6°C	Experiment	7	1.371	0.196	0.600	0.742
8°C	Experiment	7	7.650	1.093	0.830	0.592
10°C	Experiment	7	21.634	3.091	2.320	0.131
12°C	Experiment	7	70.715	10.102	1.450	0.304
14°C	Experiment	7	66.630	9.520	0.280	0.944
16°C	Experiment	7	383.040	54.720	2.580	0.104
18°C	Experiment	7	741.490	105.930	3.020	0.072
20°C	Experiment	7	2439.70	348.440	16.960	<.001

5.3.2.3 Infection frequency

Over 90% of the *P. infestans* isolates tested were able to infect the Maris Piper detached leaflets at temperatures of 10°C and above (Figure 5.4.B). At 8°C, 84% of the isolates caused infection and, interestingly, 49% of the isolates (24 out of the 49) showed signs of infection at 6°C. This ability to infect at low temperatures was not genotype-specific (Figure 5.4.C) as 10 of the 11 genotypes infected the leaflets at 6°C with only genotype 3_A2 unable to infect. In 14 of the 24 isolates infecting at 6°C, a lesion was formed on only one of the two replicate leaves. The lesions formed at 6°C were only a few mm in size but nonetheless indicate that isolates are able to infect at temperatures below 10°C.

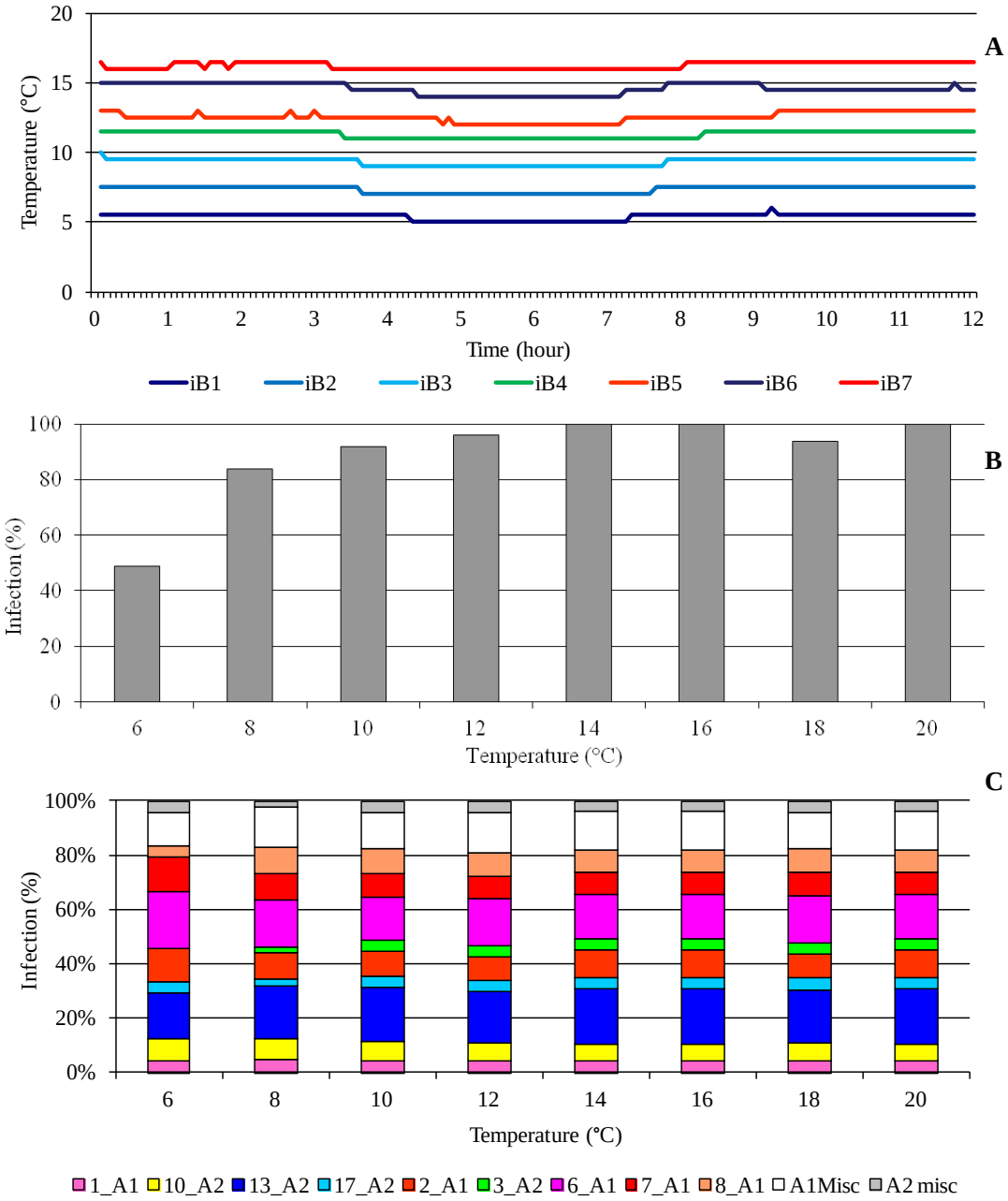


Figure 5.4 – A) Temperature data recorded the *in vivo* study on the temperature gradient plate. Twelve hours of the study is shown and data is an average of all 8 tests.

B) Percentage of UK *P. infestans* isolates able to infect detached leaflets of the potato cultivar Maris Piper at temperatures ranging from 6°C-20°C

C) Proportions of UK *P. infestans* genotypes able to infect detached leaflets of the potato cultivar Maris Piper at temperatures ranging from 6°-20°C. Coloured bars represent a genotypes. Percentages calculated by dividing the number of isolates of said genotype by the total number of isolates infected and multiplying by 100.

5.3.2.4 Incubation period

5.3.2.4.1 Isolate

No significant differences were seen between the responses of the isolates to temperatures 6°C, 8°C, and 12°C (Table 5.7; data not shown) for IP. For temperatures 10°C, 18°C and 20°C, there was a significant difference between the responses of the isolates (P=0.011, P<0.001 and P<0.001 respectively, Table 5.7). At 6°C, there was little variation seen between any of the isolates as all IP values were between 7 days and 8 days. At 8°C, 33 of the isolates had an IP of 7 days. Out of the 16 isolates that had IP values less than 7 days, five isolates belonged to genotype 13_A2, one of which had the smallest IP value. Only one isolate of genotype 6_A1 was amongst the 16 isolates. Both isolates of genotype 17_A2 had IP values that were under 7 days. At 10°C, 19 isolates had an IP value less than 7 days with a range of 3 days to 6.5 days. Five isolates of genotype 13_A2 had IP values less than 7 days. Isolate 2008_6430A had the shortest IP value and was significantly shorter than all other isolates representing genotype 13_A2; it was also significantly shorter than the majority of isolates that had IP values over 5.5 days. Two isolates representing genotype 6_A1 had IP values less than 7 days but there was no significant variation seen between them. Within other genotypes, isolate 2008_7006D, which represented genotype 2_A1, had an IP value that was significantly smaller than all other isolates representing genotype 2_A1 which were not significantly different from each other. At 12°C, isolate 2006_4388D (genotype 17_A2) had the shortest IP value, there was no significant difference between this isolate and the next 37 isolates, but its IP was smaller than the other genotype 17_A2 isolate (2006_4388C) although it was not significant. The isolates representing genotype 13_A2 showed variation within the IP value but the differences were not significant. The variation seen between the isolates representing genotype 6_A1 was greater compared to the amount of variation seen in genotype 13_A2. Isolates of

genotype 6_A1 were mostly not significantly different to one another, but isolates with the longest IP values were significantly longer than the other genotype 6_A1 isolates. At 18°C, most isolates had IPs of between 2 days and 4 days. The differences between the isolates with shortest IP values and those with intermediate values were statistically significant. The IP values for isolates of genotype 13_A2 were all 4 days or less whereas a much larger range of IP values was noted amongst isolates of 6_A1. Amongst genotype 6_A1 were isolates with both the shortest and the longest IP values; there were two distinct groups seen that were significantly different to one another; four isolates had IP value that ranged from 2.5 days to 3 days and there were another four isolates that had IP values that ranged from 4 days to 7 days. At 20°C, isolate 2006_4388D had the shortest IP value which was significantly shorter than the other genotype 17_A2 isolate. Isolates representing genotype 13_A2 had IP values that were short to intermediate; ranging from 2 days to 3.3 days although one isolate (2008_7038A) had an IP value of 7 days. The isolates with the shortest IP value were significantly different to those with the longest IP values. Isolates of genotype 6_A1 had a wide range of IP values which was again more diverse than the isolates of genotype 13_A2. There were four distinct clusters of isolates within genotype 6_A1 which were significantly different from each other and ranged from short IP values to long IP values. Genotype 6_A1 always had a shorter IP than genotype 13_A2 except at 6°C

5.3.2.4.2 Genotype

No significant differences in IP were seen between the responses of the genotypes at the temperatures 6°C, 10°C, 12°C or 20°C (Figure 5.5, Table 5.8). At 8°C and 18°C there were statistically significant differences in IP values between different genotypes (Figure 5.5, Table 5.8). At 8°C, a few of the differences between genotypes were statistically significant; for example the IP value for A2 Misc was significantly different

from 17_A2, 6_A1 and A1_Misc. Genotype 13_A2 had a shorter IP than genotype 6_A1 but it was not significantly shorter and neither was it the shortest of the 11 genotypes. Genotype 17_A2 had the shortest mean IP but due to higher variance the value was not statistically significant from the other genotypes. At 18°C, genotype 2_A2 had the shortest IP and this was significantly different to most other genotypes, including genotypes 13_A2 and 6_A1. Genotype 13_A2 had a significantly shorter IP than genotype 6_A1 although both had an intermediate IP value compared two shortest IP values which were under 3 days. Overall, genotype 13_A2 had an intermediate IP value at the lower temperatures and, as the temperature increased, the IP value was ranked fourth or above. Genotype 6_A1's position in the ranking was not consistent between the different temperatures tested.

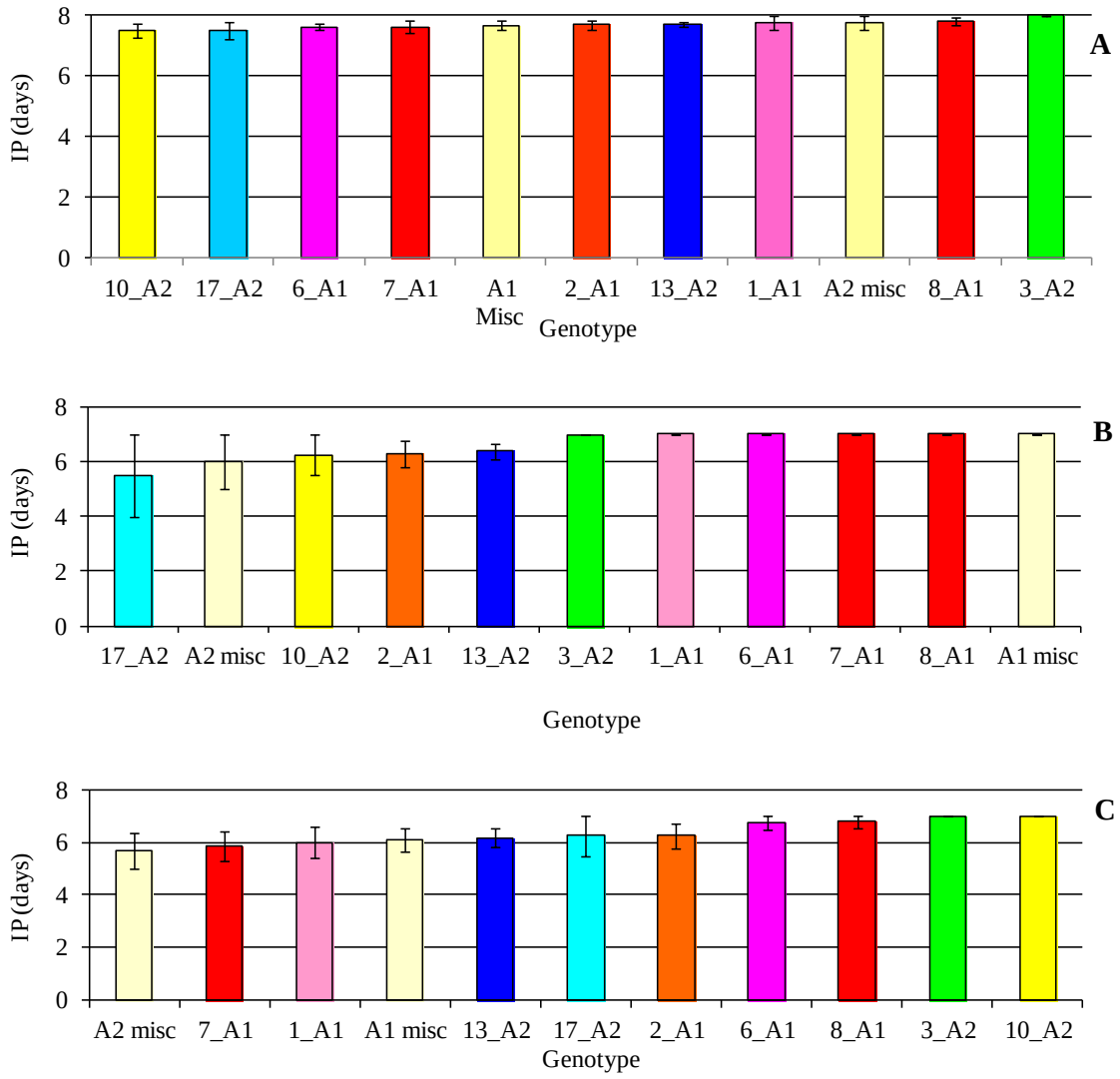


Figure 5.5 – Mean IP value of 11 UK *P. infestans* genotypes on detached leaflets of the potato cultivar Maris Piper at a range of temperatures. The error bars represent the standard errors of differences of the means (SE). Isolates used for each genotype are as follows; 1_A1 n=2, 2-A1 n=3, 3_A2 n=2, 6_A1 n=8, 7_A1 n=4, 8_A1 n=4, 10_A2 n=3, 13_A2 n=10, 17_A2 n=2, A1 Misc n=7 and A2 Misc n=2

A) 6°C SE= 10_A2 0.22, 17_A2 0.29, 6_A1 0.13, 7_A1 0.18, A1 Misc 0.14 2_A1 0.15, 13_A2 0.08, 1_A1 0.25, A2 Misc 0.25, 8_A1 0.13, 3_A2 0.00

B) 8°C SE= 17_A2 1.50, A2 Misc 1.00, 10_A2 0.75, 2_A1 0.47, 13_A2 0.29, 3_A2, 1_A1,6_A1,7_A1,8_A1 and A1 Misc 0.00

C) 10°C SE= A2 Misc 0.67, 7_A1 0.55, 1 A1 0.58, A1 Misc 0.46, 13 A2 0.35, 17_A2 0.75, 2_A1 0.49, 6_A1 0.25, 8_A1 0.22, 3 A2 and 10_A2 0.00

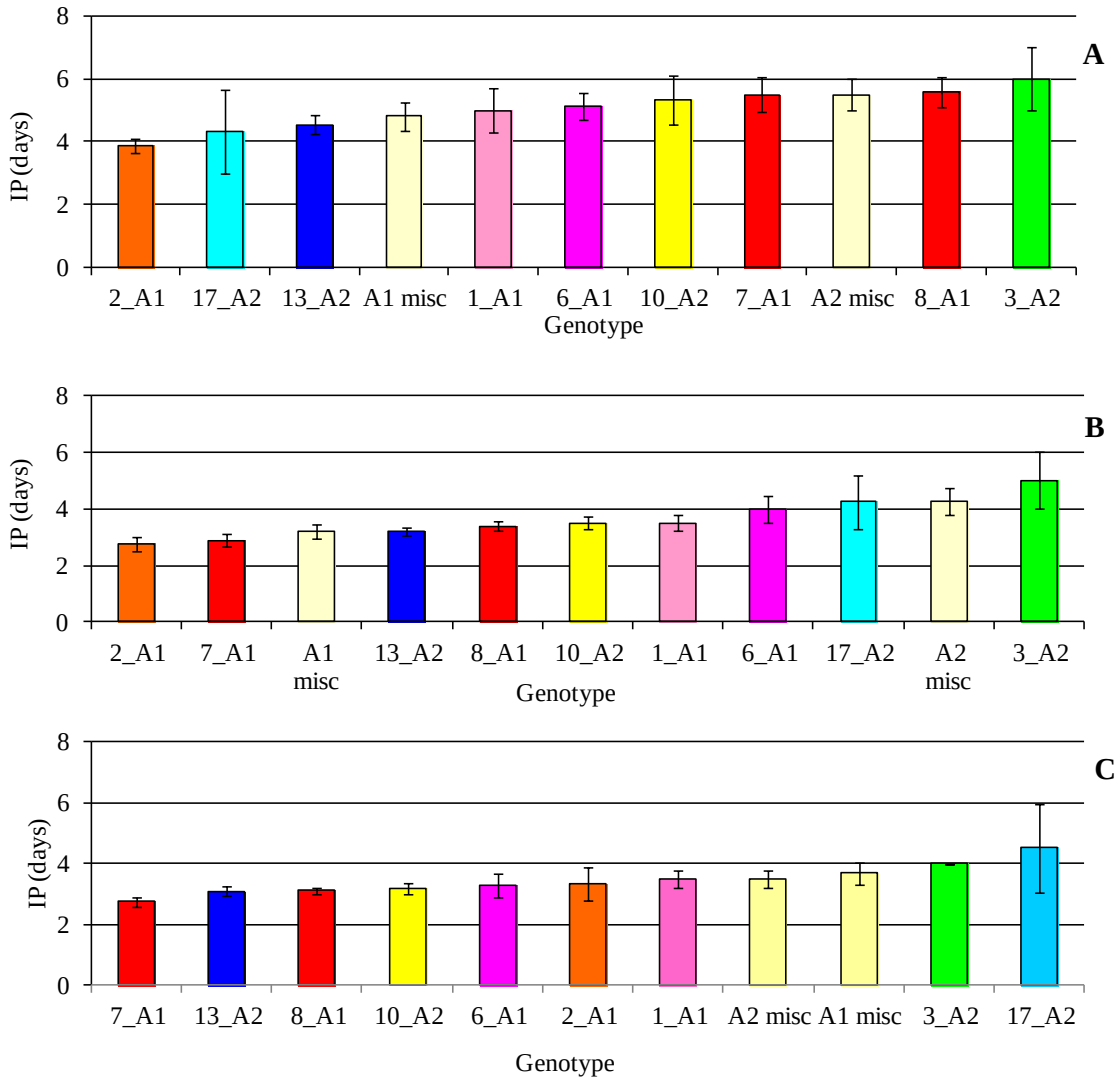


Figure 5.5 continued – Mean IP value of 11 UK *P. infestans* genotypes on detached leaflets of the potato cultivar Maris Piper at a range of temperatures. The error bars represent the standard errors of differences of the means (SE). Isolates used for each genotype are as follows; 1_A1 n=2, 2-A1 n=3, 3_A2 n=2, 6_A1 n=8, 7_A1 n=4, 8_A1 n=4, 10_A2 n=3, 13_A2 n=10, 17_A2 n=2, A1 Misc n=7 and A2 Misc n=2

A) 12°C SE= 2_A1 0.23, 17_A2 1.33, 13_A2 0.30, A1 Misc 0.44, 1 A1 0.71, 6_A1 0.45 10_A2 0.76, 7_A 0.57, A2 Misc 0.50, 8_A1 0.48 and 3 A2 1.00

B) 18°C SE= 10_A2 2.83, 7_A1 3.00, 1_A1 3.25, 13_A2 3.67, 2 A1 3.89, 3 A2 4.00 8_A1 4.00, 17_A2 4.25, A1 Misc 4.36, 6 A1 4.40 and A2 Misc 5.00

C) 20°C 7_A1 0.16, 13_A2 0.17, 8_A1 0.10, 10_A2 0.17, 6_A1 0.38, 2_A1 0.53, 1_A1 0.29, A2 Misc 0.29, A1 Misc 0.36, 3_A2 0.00, 17_A2 1.44

Table 5.7 – Descriptive statistics for *in vivo* temperature investigations; testing how temperature affects IP values of *P. infestans* isolates on Maris Piper detached leaflets. Temperatures 14°C and 16°C were not included as variation between the standard isolates' performance in different tests were significant (values shown in Appendix v)

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
6°C	IP	48	12.62	0.26	1.45	0.084
8°C	IP	48	32.16	0.84	0.98	0.529
10°C	IP	48	88.87	2.01	2.07	0.011
12°C	IP	48	138.36	3.00	1.61	0.057
18°C	IP	48	111.22	2.47	4.94	<.001
20°C	IP	48	124.42	2.59	5.1	<.001

Table 5.8 – Descriptive statistics for *in vivo* temperature investigations; testing how temperature affects IP values of *P. infestans* genotypes on Maris Piper detached leaflet. Temperatures 14°C and 16°C were not included as variation between the standard isolates' performance in different tests were significant

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
6°C	IP	10	1.01	0.10	0.44	0.921
8°C	IP	10	17.18	1.71	2.33	0.023
10°C	IP	10	14.33	1.43	0.93	0.511
12°C	IP	10	30.14	3.01	1.30	0.244
18°C	IP	10	28.89	2.89	2.70	0.007
20°C	IP	10	14.08	1.40	1.08	0.386

5.3.2.5 Lesion size

5.3.2.5.1 Isolate

All lesion sizes were scored after 7 days. The lesions at 6°C were extremely small but still measurable and as the temperature increased so did the size of the lesions. Final lesion size was used to make comparisons between genotypes. Lesion size ranged from 3.65 mm^2 at 6°C to 49.97 mm^2 at 20°C.

A significant difference between the responses of the isolates was seen for most temperatures (Table 5.9). At 6°C, there was no lesion recorded for 25 isolates. Four isolates of genotype 13_A2 formed lesions; the mean lesion size formed by isolate 2008_6102A was significantly larger than those caused by all other isolates. 13_A2 isolates 2008_6430A, 07_39 and 2006_3928A also formed lesions that were within the same size range of those caused by the four isolates of 6_A1 (Appendix iv). At 10°C, the majority of the genotype 13_A2 isolates had large to intermediate sized lesions and clustered into groups of two to three isolates (most of which were not significantly different); three genotype 13_A2 isolates were amongst the four largest lesion sizes. Isolates representing genotype 6_A1 formed large to intermediate sized lesions most of which were not significantly different to one another. Two isolates representing genotype 17_A2 (2006_4388C and 2008_4338D) were significantly different to one another with mean lesion sizes of 0.74 mm^2 and 3.90 mm^2 respectively. At 12°C, isolates that represented genotype 13_A2 had a lesion size that was of a high to intermediate level; one isolate (2008_7038A) failed to grow at all. Most isolates were not significantly different to one another. Isolate 2008_6090A and 2008_6426A had the largest lesion sizes and were significantly different to most isolates that represented genotype 6_A1; these isolates formed lesions that were intermediate to small in size. At 14°C, isolates that represented genotype 13_A2 fell in to high/intermediate lesion sizes and these were not significantly different to one another. The two largest mean lesion

sizes belonging to isolates 2008_7034A and 2008_6090A (genotype 6_A1) were significantly larger than the isolates with intermediate to small lesions. Four out of the eight isolates that represent genotype 6_A1 formed some of the largest lesions, two isolates had intermediate lesion sizes and two formed small lesions. At 16°C, the majority of the isolate representing genotype 13_A2 had some of the largest lesion sizes. Isolates representing genotype 6_A1 had the largest range of lesion size possible with isolates representing genotype 6_A1 forming both the largest (isolate 2008_7034E) and the smallest lesions (isolate 2008_6498A). More significant variation was seen within the isolates of genotype 6_A1 than genotype 13_A2. The three isolates that produced the smallest lesion sizes were significantly smaller than the rest of the isolates representing genotype 6_A1. At 18°C, the lesions generated by genotype 13_A2 isolates 2008_7038A, 07_39 and 2008_6250D were significantly smaller than the other seven isolates that represented genotype 13_A2. Isolate 2008_6530C and 2008_6050B lesions were significantly larger than isolates with intermediate lesion sizes. Isolates representing genotype 6_A1 had a broad range of variation with no obvious clustering of isolates. Isolate 2006_4388D formed the largest lesions out of all the isolates tested at 18°C, significantly larger than those of isolate 2008_4388C, which is the same genotype.

5.3.2.5.2 Genotype

There were no significant differences in the genotypic response to any temperature for LS (Figure 5.6, Table 5.10). Although the amount of variation seen between the genotypes meant that there were no significant differences seen for lesion size, the genotypes did have different values with genotype A2 Misc having the largest lesions out of the 11 genotypes tested. Genotype 13_A2 was ranked third for lesion size; genotype 6_A1 had the smallest mean lesion size. The only genotype that did not infect was genotype 3_A2. At 8°C, 13_A2 formed the largest lesions after 7 days closely

followed genotype 1_A1 (Figure 5.6). At 10°C, genotype 13_A2 produced the second largest mean lesion size after seven days. As the temperature increased, the mean lesion size of genotype 13_A2 isolates was in a more intermediate position. The mean sizes of lesions formed by genotype 6_A1 isolates were very similar to genotype 13_A2 at all temperatures except 8°C where genotype 6_A1 produced smaller lesions than 13_A2 and at 12°C where genotype 6_A1 isolate lesions were, on average, larger than 13_A2's. The ranking order between the genotype for each temperature varied. For example, 13_A2 did not have a consistent ranking as it did with the IP values, but it did lie within the top half of the genotypes throughout all temperatures (data not shown). Genotype 6_A1 ranged from being ranked third to sixth depending on the temperature. Genotype 2_A1 isolates formed consistently small lesions size at all temperatures. In general, lesion size increased with temperature but, as the experiment only went to 20°C it was not possible to determine the temperature at which the growth of *P. infestans* would be hindered.

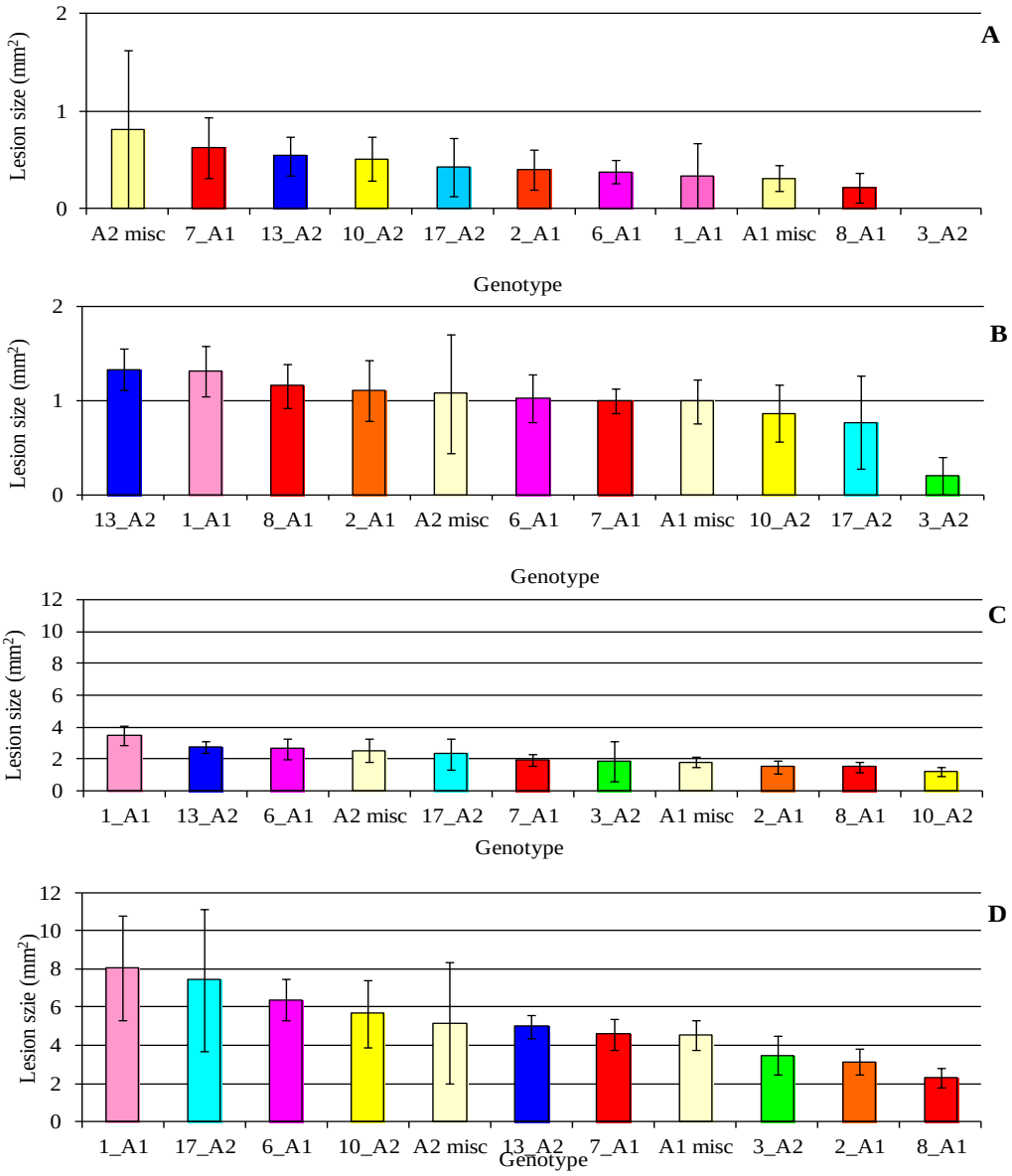


Figure 5.6 – Mean final mean lesion size for 11 UK *P. infestans* genotypes on detached leaflets of the potato cultivar Maris Piper at a range of different temperatures. The error bars represent the standard errors of differences of the means (SE). Isolates used for each genotype are as follows; 1_A1 n=2, 2-A1 n=3, 3_A2 n=2, 6_A1 n=8, 7_A1 n=4, 8_A1 n=4, 10_A2 n=3, 13_A2 n=10, 17_A2 n=2, A1 Misc n=7 and A2 Misc n=2

A) 6°C SE= A2 Misc 0.81, 7_A1 0.31, 13_A2 0.19, 10_A2 0.23, 17_A2 0.30, 2_A1 0.31, 6_A1 0.12, 1_A1 0.33, A1 Misc 0.13, 8_A1 0.15 and 3_A2 0.00

B) 8°C SE= 13 A2 0.22, 1_A1 0.27, 8_A1 0.24, 2_A1 0.32, A2 Misc 0.63, 6_A1 0.25, 7_A1 0.13, A1 Misc 0.24, 10_A2 0.30, 17_A2 0.49 and 3_A2 0.20

C) 10°C SE= 1_A1 0.62, 13 A2 0.38, 6_A1 0.64, A2 Misc 0.75, 17_A2 0.94, 7_A1 0.37, 3_A2 13.28, A1 Misc 0.31, 2_A1 0.39, 8_A1 0.30 and 10 A2 0.28

D) 12°C 1_A1 2.71, 17_A2 3.70, 6_A1 1.11, 10_A1 1.74, A2 Misc 3.18, 13 A2 0.62, 7_A1 0.79, A1 Misc 0.76, 3_A2 1.01, 2_A1 0.69 and 8_A1 0.49

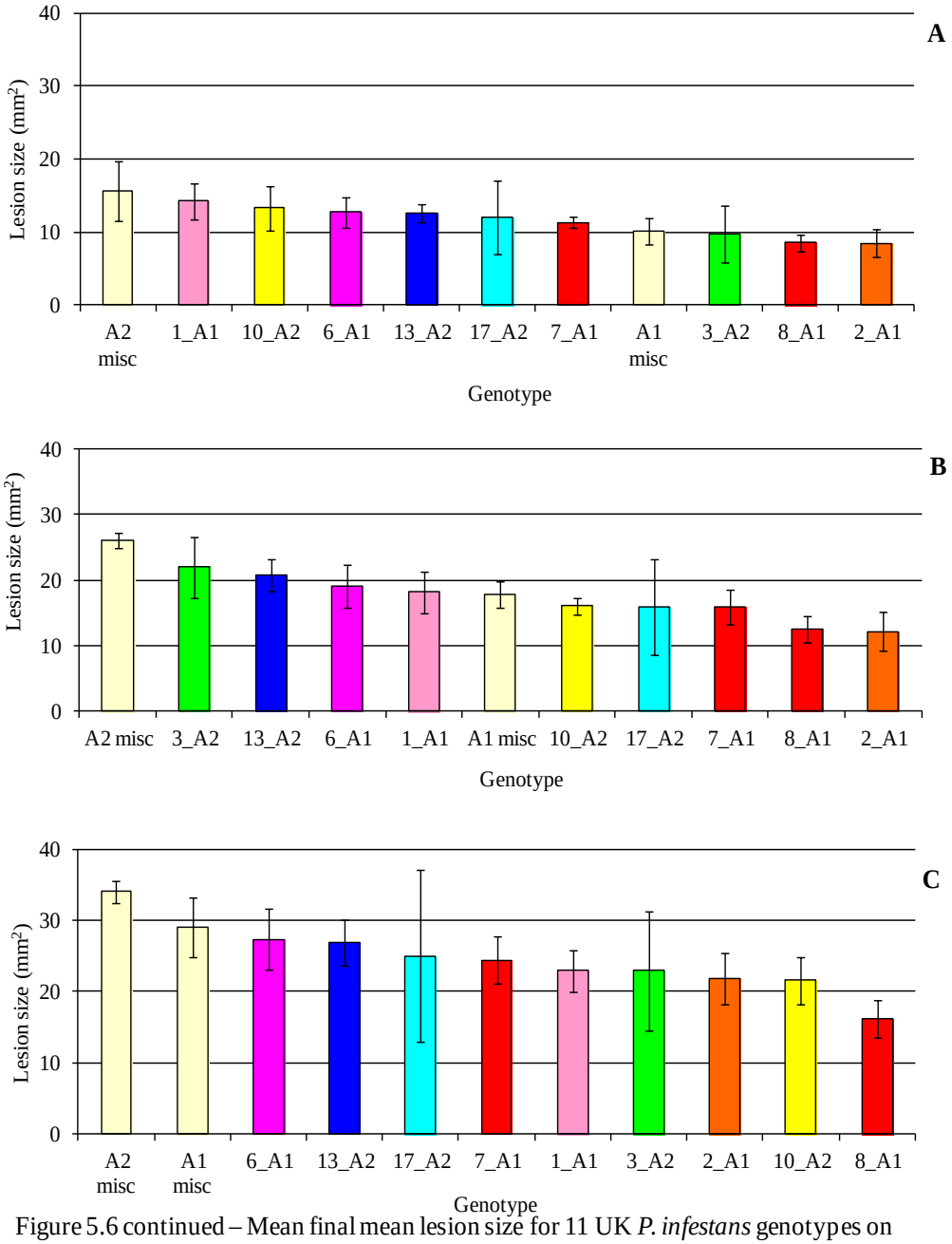


Figure 5.6 continued – Mean final mean lesion size for 11 UK *P. infestans* genotypes on detached leaflets of the potato cultivar Maris Piper at a range of different temperatures. The error bars represent the standard errors of differences of the means (SE). Isolates used for each genotype are as follows; 1_A1 n=2, 2-A1 n=3, 3_A2 n=2, 6_A1 n=8, 7_A1 n=4, 8_A1 n=4, 10_A2 n=3, 13_A2 n=10, 17_A2 n=2, A1 Misc n=7 and A2 Misc n=2

A) 14°C SE= A2 Misc 4.08, 1 A1 2.42, 10_A2 3.01, 6_A1 2.05, 13 A2 0.31, 17 A2 5.02, 7_A1 0.77, A1 Misc 1.75, 3_A2 3.89, 8_A1 1.12 and 2_A1 1.94

B) 16°C SE= A2 Misc 1.09, 3 A2 4.65, 13 A2 2.45, 6_A1 3.27, 1 A1 3.18, A1 Misc 2.02, 10_A2 1.22, 17 A2 7.27, 7_A1 2.60, 8_A1 2.06 and 2_A1 3.04

C) 18°C SE= A2 Misc 1.52, A1 Misc 4.17, 6_A1 4.31, 13_A2 3.17, 17 A2 12.13, 7_A1 3.30, 1_A1 2.85, 3_A2 8.45, 2_A1 3.53, 10_A2 3.31 and 8_A1 2.61

Table 5.9 – Descriptive statistics for *in vivo* temperature investigations; testing how temperature affects lesion size of *P. infestans* isolates on Maris Piper detached leaflet. Temperature 20°C was not included as variations between the standard isolate were significant (values shown in Appendix v).

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
6°C	LS	48	49.85	1.03	8.78	<.001
8°C	LS	48	55.66	1.16	1.63	0.052
10°C	LS	48	152.64	3.47	2.60	0.002
12°C	LS	48	836.23	17.42	2.14	0.005
14°C	LS	48	3119.40	64.99	3.66	<.001
16°C	LS	48	8241.43	171.70	8.07	<.001
18°C	LS	48	15716.56	334.39	11.23	<.001

Table 5.10 – Descriptive statistics for *in vivo* temperature investigations; testing how temperature affects lesion size of *P. infestans* genotype on Maris Piper detached leaflet. Temperature 20°C was not included as variations between the standard isolate were significant

Temperature	Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
6°C	LS	10	2.80	0.28	0.50	0.883
8°C	LS	10	1.15	0.12	1.89	0.068
10°C	LS	10	34.88	3.44	1.38	0.207
12°C	LS	10	212.36	21.24	1.81	0.070
14°C	LS	10	376.97	37.70	0.96	0.487
16°C	LS	10	1224.42	122.44	1.38	0.203
18°C	LS	10	1654.80	165.50	0.96	0.481

5.3.3 Relationships between the *in vitro* and *in vivo* growth

A ranking analysis was used to determine whether there was a relationship between the mean *in vitro* growth on agar and *in vivo* growth on leaves of each genotype (Figure 5.7). In three cases; at 10°C genotype 1_A1 and at 20°C both 10_A2 and A2 Misc, *in vitro* and *in vivo* ranks are identical. However, in general, *in vitro* and *in vivo* growth was not comparable. Genotype 3_A2, for example, did not form the largest lesions at any temperature *in vivo* but had the largest *in vitro* colonies. Temperature made a difference when comparing *in vivo* and *in vitro* results For example, at 20°C A2 Misc had the same rank for both *in vivo* and *in vitro* but at 10°C the ranks are greatly different between *in vivo* and *in vitro*. This was seen for genotype 1_A1, 3_A2, 7_A1, 10_A2 and A1 Misc. Genotype 13_A2 had similar ranks for the temperatures but very different ranks between the tests. Genotype 6_A1 and 17_A2 were inconsistent for both temperatures and tests.

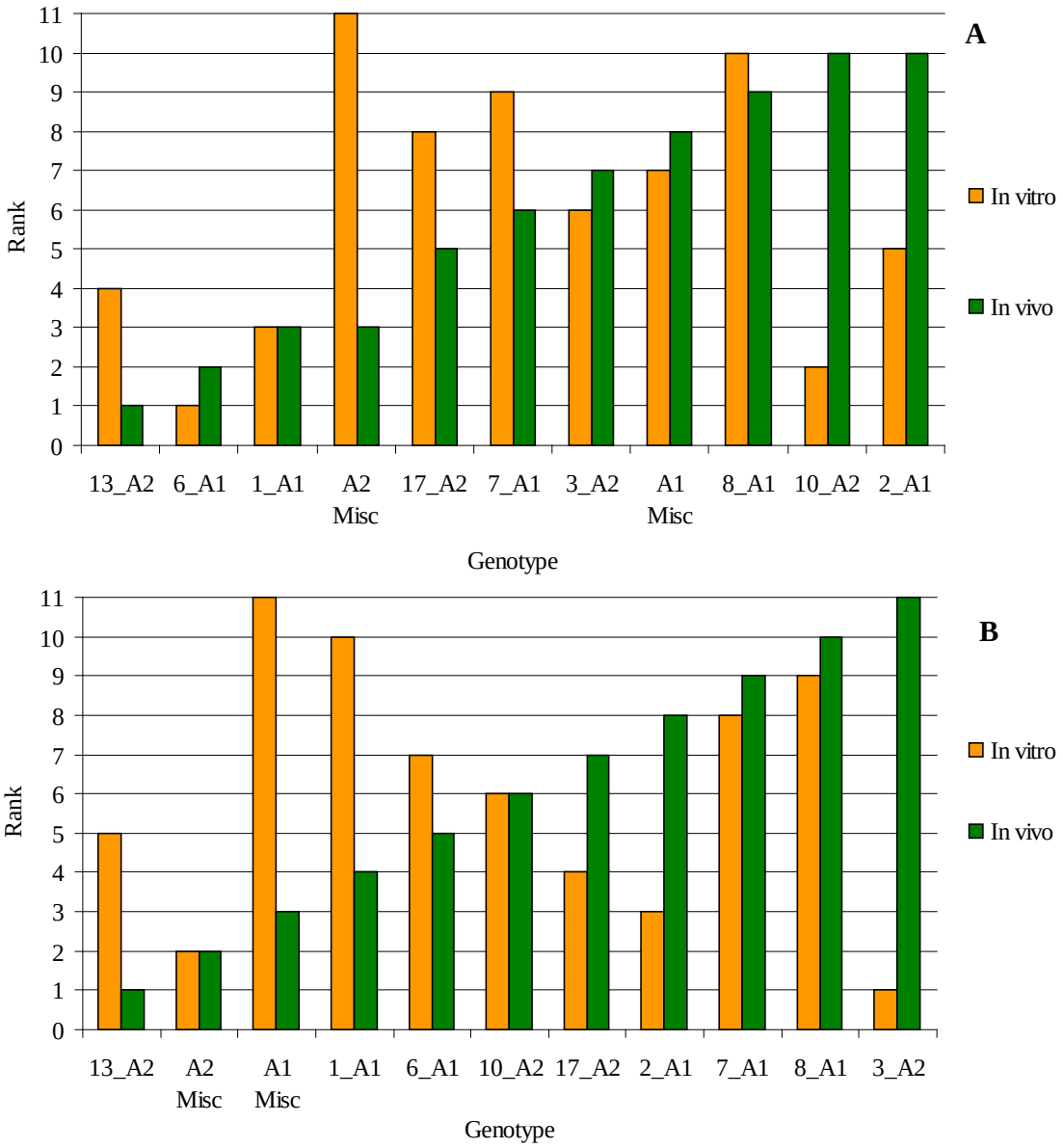


Figure 5.7 – Ranked order of the *in vitro* and *in vivo* mean colony/lesion size for each genotype to assess comparability

A) *In vitro* and *in vivo* at 10°C B) *In vitro* and *in vivo* at 20°C

5.3.4 In vivo growth with diurnal temperature patterns

5.3.4.1 IP

Temperature had a significant effect on the IP of the four isolates representing four different genotypes (*P* <.001, Figure 5.8, Table 5.11). As expected, IP reduced with increasing temperatures with the longest mean IP value of 7.1 days at mean temperatures of 5°C significantly longer than values of around 3 days at mean temperatures of 15°C, 16°C and 17°C (Figure 5.14.A). The IP at 8°C was not significantly different to the IP at 10°C.

Genotype had a significant effect on IP (*P* <.001, Figure 5.8, Table 5.11). Over all temperatures, genotype 2_A1, 13_A2 and 6_A1 had a significantly shorter mean IP than 17_A2 (5.4 days). Genotype 17_A2 had the longest IP at most mean temperatures from 8°C upwards. Genotype 13_A2 had similar or a slightly longer IP at most mean temperatures compared to 6_A1 except between the temperatures 8°C–11°C (Figure 5.8).

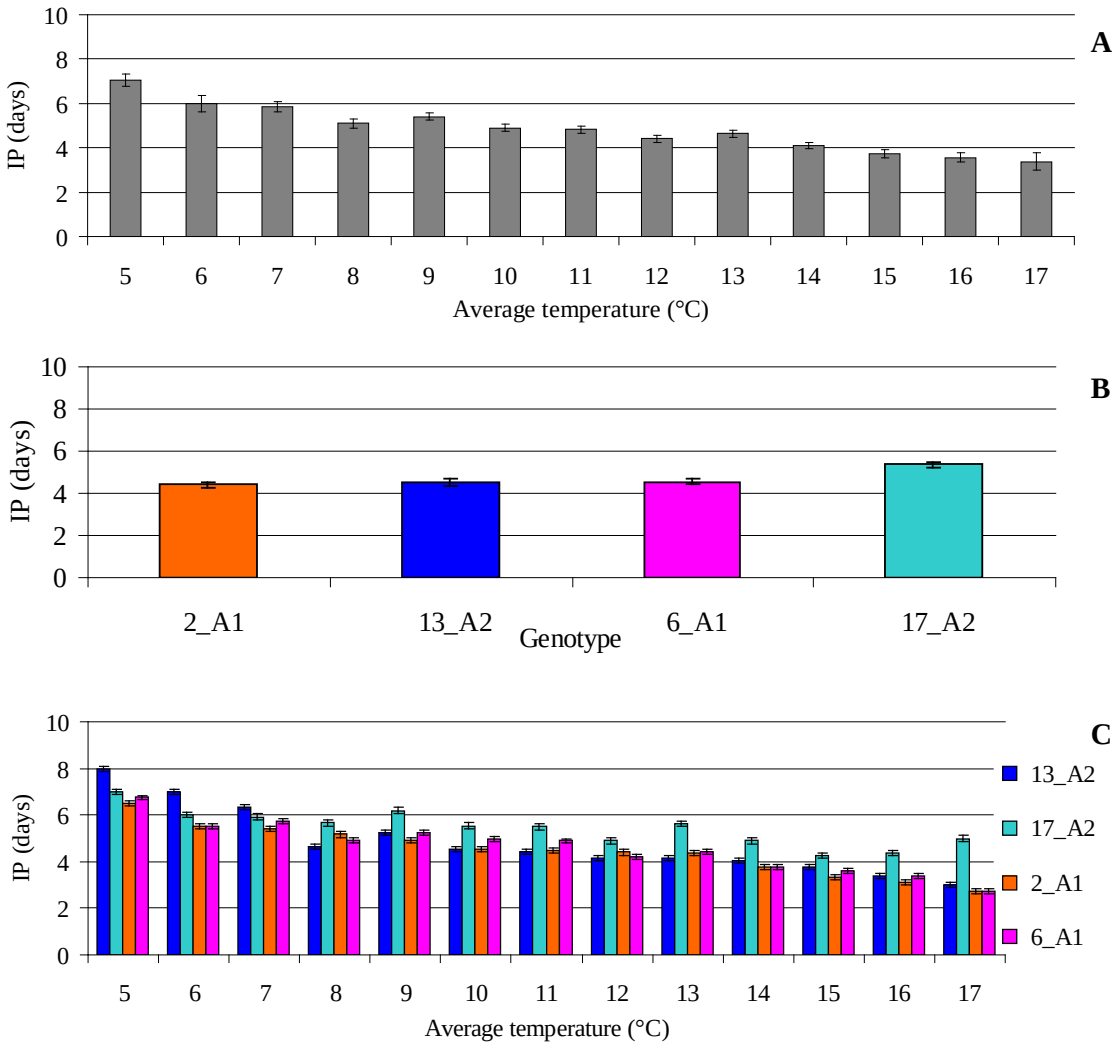


Figure 5.8 – Mean IP values for four UK *P. infestans* genotypes on Maris Piper detached leaflets that were incubated on a temperature gradient plate set to generate different diurnal temperature regimes. Data for 13 mean temperatures are shown to indicate the range of responses. The errors bars represent the standard errors of differences of the means (SE)

A) Mean IP value for each mean temperature meaned over genotype. SE= 5°C 0.28, 6°C 0.36, 7°C 0.23, 8°C 0.20, 9°C 0.17, 10°C 0.15, 11°C 0.15, 12°C 0.16, 13°C 0.15, 14°C 0.13, 15°C 0.19, 16°C 0.20, 17°C 0.38

B) Mean IP values for each genotype meaned over all temperatures. SE=0.14

C) Mean IP values for each genotype at all of the meaned temperature combinations. SE= 13_A2 0.10, 17_A2 0.12, 2_A1 0.11, 6_A1 0.10.

5.3.4.1.1 Day and night temperature combinations

Significant differences in IP were observed between the responses of isolates to different day and night temperature combinations (P <.001, Figure 5.9, Table 5.11). Infection was noted at all temperature combinations. The shortest IP was seen at the combinations around 17°C/13°C and the longest was at the combination of 5°C/5°C. Combinations with low temperatures produced longer IPs even in combination with a higher temperature, for example, isolates had a mean IP of 5.5 days when incubated 15°C/5°C (i.e. 15°C for 16 daylight hours and 5°C for 8 night time hours) compared to the 15°C/13°C which had an IP value of 3.8 days. Having a low temperature for the 16 hour daylight period did extend the IP but when it is coupled with a higher night time temperature the 8 hour warmer period shortened the IP; for example, at the combination 6°C/8°C the IP value was 6.3 days whereas for the combination 6°C/14°C the IP value decreased to 4.8 days.

Genotype 13_A2 did not infect at 5°C/5°C but all other genotypes did. 13_A2 had a shorter IP value when the temperature combinations contained a low temperature. For example, for the six combinations with 8°C as the day time temperature it was seen that 13_A2 had a lower IP for all but two (8°C/11°C and 8°C/13°C; Figure 5.14.A and Figure 5.14.B). Similarly, genotype 13_A2 had short IP values at the temperature combinations with 11°C and 12°C as the day time temperature; at every combination genotype 13_A2 had the shortest IP when compared to the other three genotypes. Whereas, of the seven temperature combination with 16°C and 17°C for 16 hours, genotype 13_A2 only had the shortest IP once, at 16°C/16°C (Figure 5.14.D). It could be seen that with a day temperature of 15°C and night time temperatures of 5°C or 7°C the IP values for genotype 13_A2 were shorter than 6_A1, 2_A1 and 17_A2. When the night time temperature was higher for example 15°C/13°C and 15°C/15°C, the IP value

for genotype 13_A2 was longer than the other genotypes. This same pattern can be seen for the temperature combinations of 13°C/5°C, 13°C/7°C and 13°C/11°C.

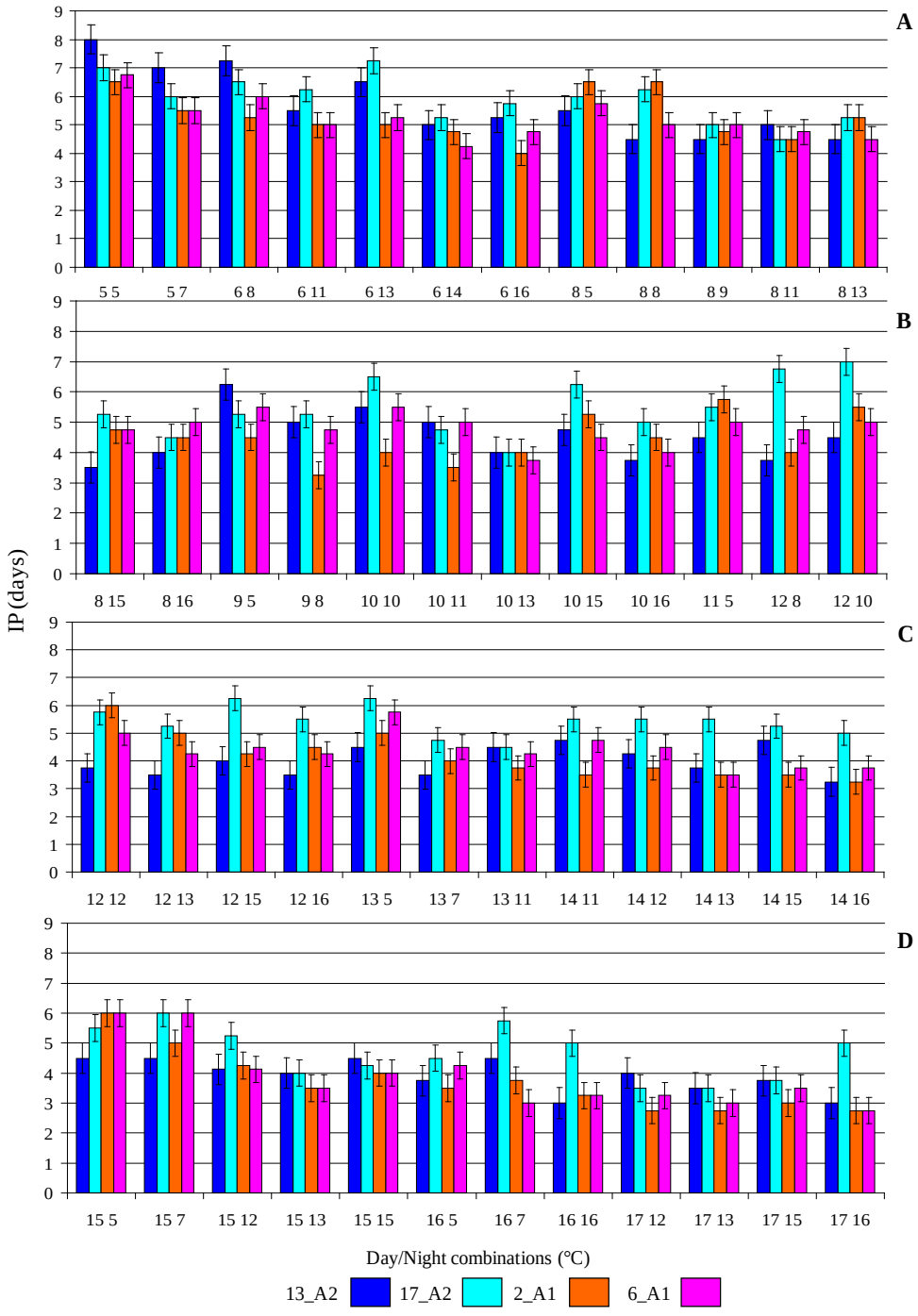


Figure 5.9 – Mean IP values for four UK *P. infestans* genotypes on Maris Piper detached leaflets that were incubated on a temperature gradient plate to provide different day and night temperature combinations. The errors bars represent the standard errors of differences of the means (SE). SE= 0.51

A) Mean IP values for temperature combinations ranging from 5°C/5°C to 8°C/13°C

B) Mean IP values for temperature combinations ranging from 8°C/15°C to 12°C/10°C

C) Mean IP values for temperature combinations ranging from 12°C/12°C to 14°C/16°C

D) Mean IP values for temperature combinations ranging from 15°C/5°C to 17°C/16°C

5.3.4.1.2 Accumulated day degrees

The heat plots show the mean IP value at each of the temperature combinations and the colour scale shows the broad differences between the genotypes (heat plot discussed in Chapter 4.3.1.3). Genotype 17_A2 had longest IP at most of the points in the temperature gradient plate (Figure 5.10). Genotype 13_A2 had a short IP value (under 5 days) for the middle to low temperatures points. Genotype 6_A1 and 2_A1 had very short IP values at the very high temperature combinations and had longer IP values than genotype 13_A2 in the mid-range temperatures.

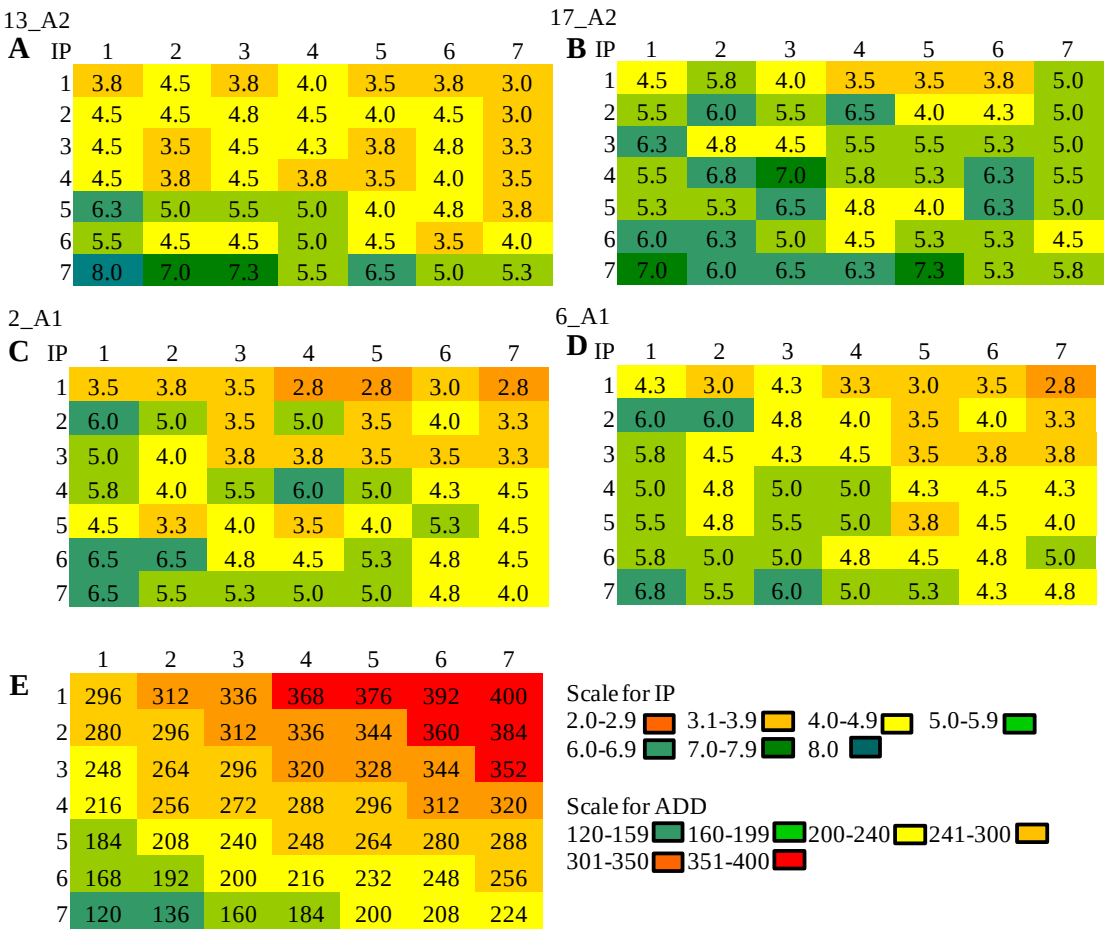


Figure 5.10 – Mean IP values for four UK *P. infestans* genotypes on Maris Piper for each point on the temperature gradient plate. Each point represents a different temperature combination.
A) Genotype 13_A2
B) Genotype 17_A2
C) Genotype 2_A1
D) Genotype 6_A1
E) The accumulated day degrees (ADD) for each point on the temperature gradient plate.

5.3.4.2 LP

Temperature had a significant effect on the LP values (P <.001, Figure 5.11, Table 5.12). At 5°C there was no sporulation. At higher temperatures, genotypes had shorter LPs than at the lower temperatures, for example, the shortest LP was at the mean temperature of 17°C; significantly shorter than the IP values at means of 16°C, 14°C and below. The LP values at mean temperatures of 6°C and 7°C were not significantly different to one another but were significantly different to those at 10°C and above.

The isolates of different genotypes had significantly different LPs (P = 0.006, Figure 5.11, Table 5.12). Genotype 2_A1, 13_A2 and 6_A1 were not significantly different to one another but genotype 17_A2 had a significantly longer LP (Figure 5.11.B). Genotype 17_A2 had the longest LP at mean temperatures from 13°C to 17°C. The only temperature where genotype 17_A2 had the shortest LP was at 6°C. Genotype 6_A1 had the shortest LP value at 17°C; it had a very similar LPs to genotypes 13_A2 and 2_A1 at the other mean temperatures, for example, at 8°C, 11°C, 13°C and 15°C. It was only at the mean temperatures above 15°C that all genotypes sporulated before 7 days post inoculation. Below the mean temperature of 10°C, sporulation became sporadic with some replicates of each genotype failing to sporulate.

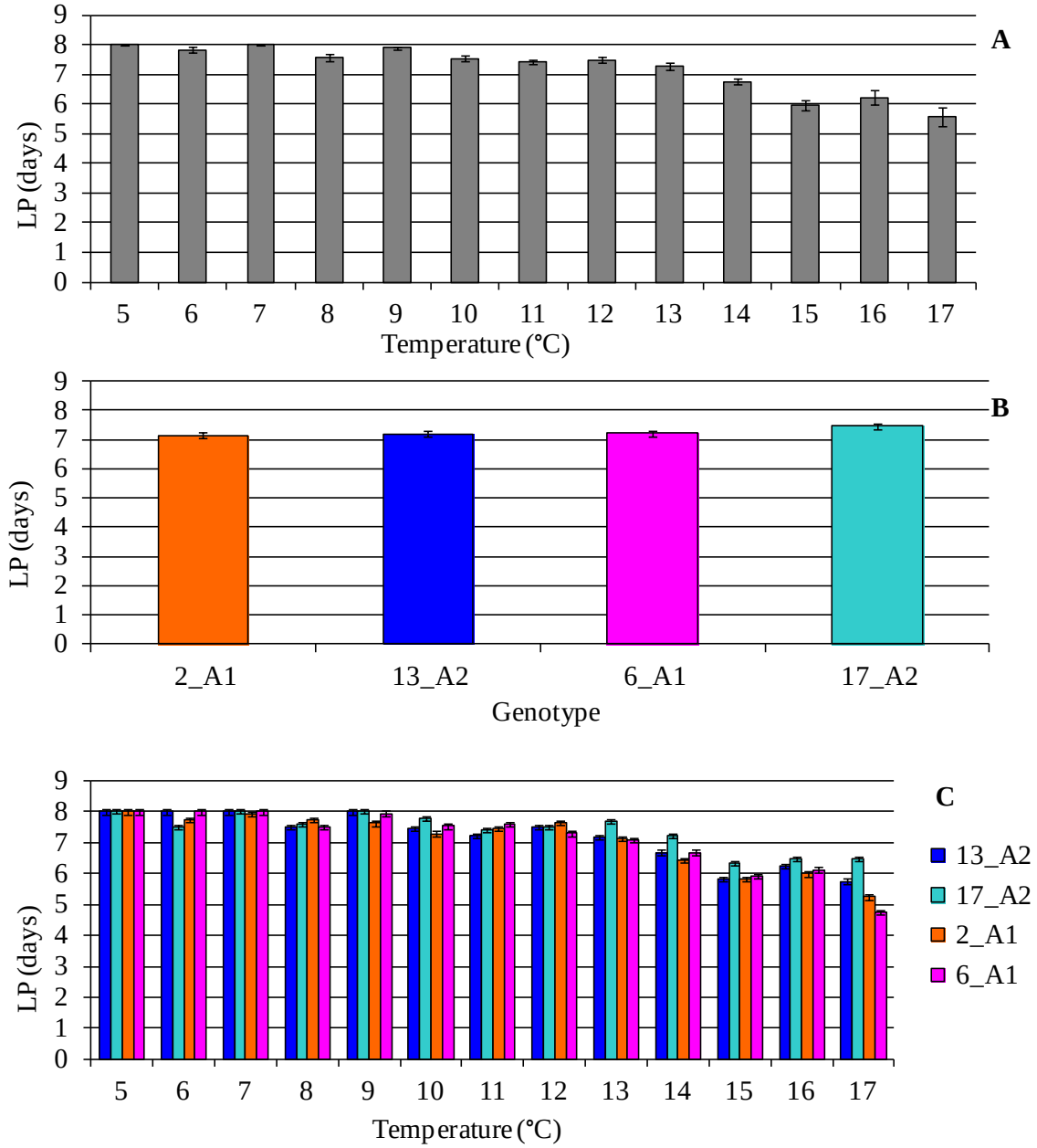


Figure 5.11 – Mean LP values for four UK *P. infestans* genotypes on Maris Piper detached leaflets that were incubated on a temperature gradient plate set to generate different diurnal temperature regimes. Data for 13 mean temperatures are shown to indicate the range of responses. The errors bars represent the standard errors of differences of the means (SE)
A) Mean LP for each mean temperature meaned over genotype. SE= 5°C 0.00, 6°C 0.10, 7°C 0.02, 8°C 0.12, 9°C 0.05, 10°C 0.10, 11°C 0.09, 12°C 0.11, 13°C 0.11, 14°C 0.11, 15°C 0.18, 16°C 0.23, 17°C 0.30
B) Mean LP values for each genotype meaned over all temperature. SE=0.09
C) Mean LP values for each genotype at all of the meaned temperature combinations. SE= 13_A2 0.08, 17_A2 0.07, 2_A1 0.08, 6_A1 0.08

5.3.4.2.1 Day and night combinations

A significant effect of day and night combinations was seen on the LP values of each genotype (Figure 5.12, Table 5.12). No sporulation was seen for the temperature combinations 5°C/5°C and 9°C/5°C for any genotype. Many temperature combinations had a LP value of 7.0 days and above, indicating that not all the genotypes and replicates sporulated (Figure 5.12). Temperature combinations that included low temperatures had longer LP values when compared to temperature combinations with a higher temperature which had the shortest LP values.

Genotype 13_A2 did not sporulate until the temperature combination reached 8°C/8°C (Figure 5.17.A). Even at the higher temperature combinations of 9°C/5°C, 9°C/8°C, 10°/10°C, 10°C/15°C, 10°C/16°C and 13°/5°C there was no sporulation for genotype 13_A2. Genotype 13_A2 had the second largest number of non-sporulating lesions out of the genotypes, genotype 17_A2 had the most, genotype 2_A1 had the least and 6_A1 had the second least. Genotype 13_A2 had shorter LP values when the temperature combinations had a low temperature in them; 8°C/8°C, 8°C/11°C, 8°C/13°C, 11°C/5°C 12°C/12°C, 12°C/13°C, 12°C/16°C, 15°C/5°C and 15°C/7°C The highest temperature combination was 17°C/16°C and genotype 6_A1 had the shortest LP as it did at the temperature combination 17°C/13°C.

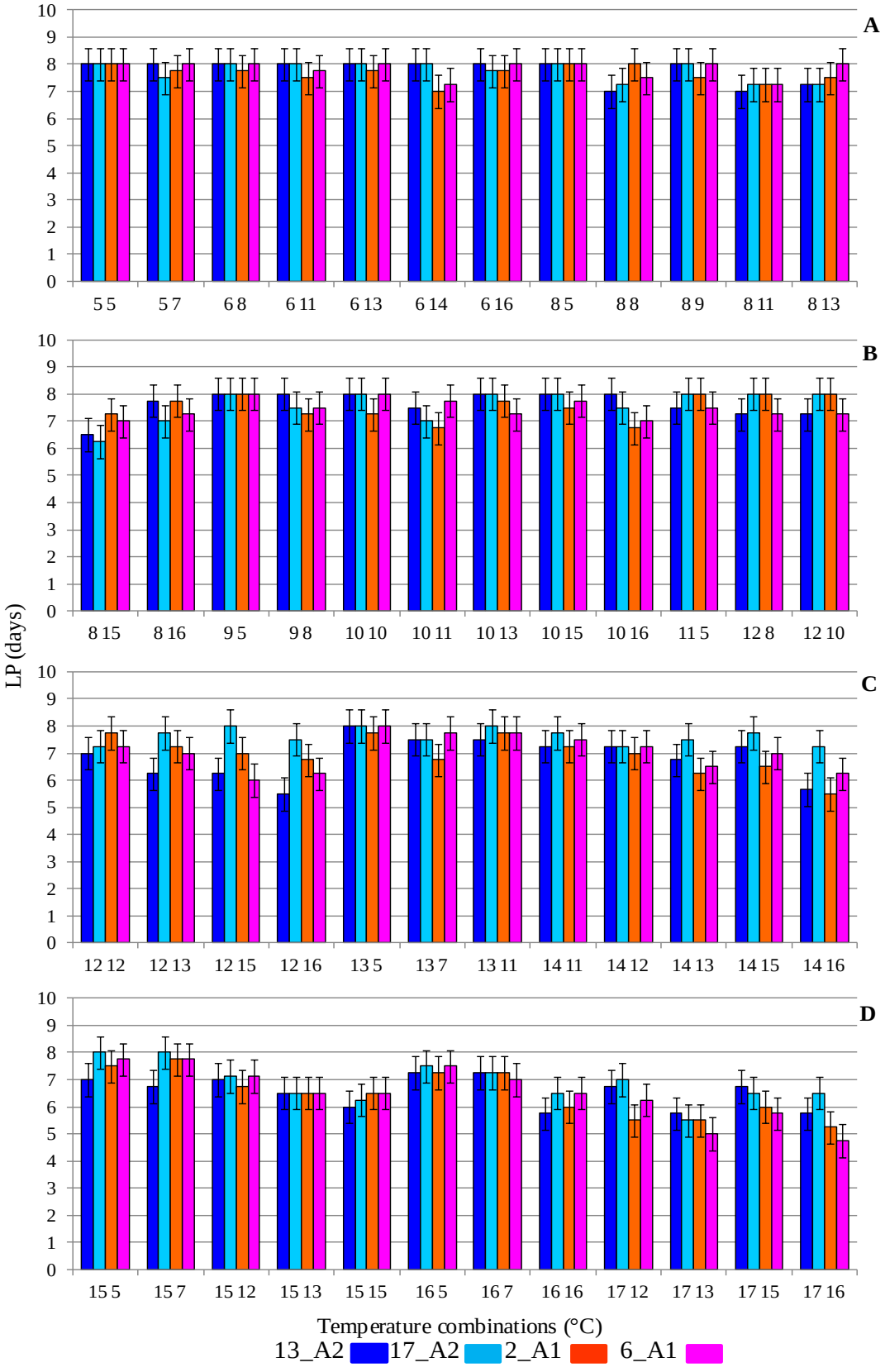


Figure 5.12 – Mean LP for four UK *P. infestans* genotypes on Maris Piper detached leaflets that were incubated on a temperature gradient plate to provide different day and night temperature combinations. The errors bars represent the standard errors of differences of the means (SE). SE= 0.30

A) Mean LP values for temperature combinations ranging from 5°C/5°C to 8°C/13°C

B) Mean LP values for temperature combination ranging from 8°C/15°C to 12°C/10°C

C) Mean LP values for temperature combinations ranging from 12°C/12°C to 14°C/16°C

D) Mean LP values for temperature combinations ranging from 15°C/5°C to 17°C/16°C

5.3.4.2.2 Accumulated day degrees

Overall, the majority of the LP values were between 7.0 days and 8.0 days. Only at the higher temperatures did the LP values go below 7.0 days and occasionally in the mid range temperatures for genotype 13_A2 and 2_A1. Genotype 13_A2 did not sporulate at the cooler temperatures but performed better than the other genotypes in the mid range temperature at most points (Figure 5.13). All genotypes had the shortest LP values at the higher ADD levels. The shortest LP value for 13_A2 was down the right hand side of the gradient plate (Figure 5.18.A) showing that genotype 13_A2 sporulated faster with night temperatures that were cooler when the day temperature was 20°C. Genotype 2_A1 had a shorter LP than all other genotypes for the ADD of 136-224.

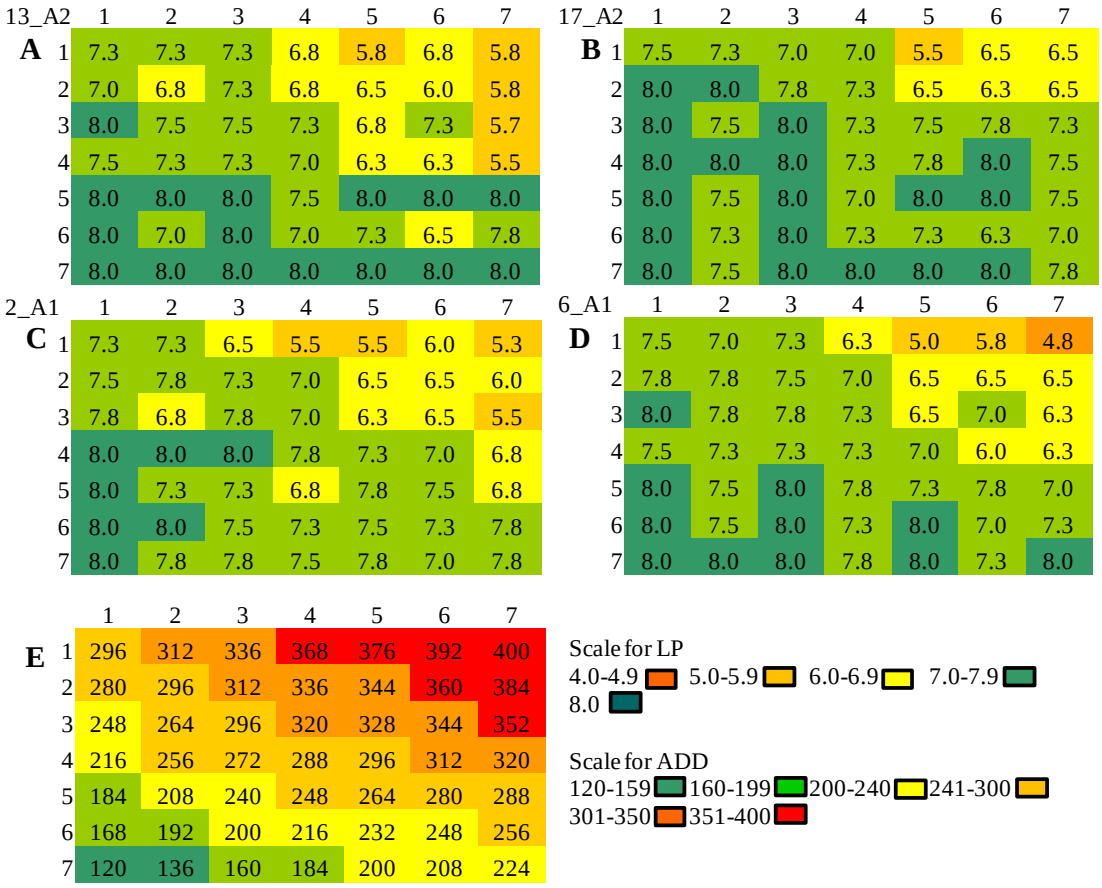


Figure 5.13 – Mean LP values for four UK *P. infestans* genotypes on Maris Piper for each point on the temperature gradient plate. Each point represents a different temperature combination.
A) Genotype 13_A2
B) Genotype 17_A2
C) Genotype 2_A1
D) Genotype 6_A1
E) The accumulated day degrees (ADD) for each point on the temperature gradient plate.

5.3.4.3 Lesion expansion

Temperature had a significant effect on lesion size (P <0.001, Figure 5.14, Table 5.13). Genotypes grown at the higher temperatures had larger lesions than at lower temperatures, for example, at the average temperature of 17°C the lesion size was on average 28.86 mm^2 and for 10°C the average lesion size was 4.32 mm^2. The variation between the genotypes gets larger as the temperatures increase (Figure 5.14). At 16°C and 17°C the lesions were very similar in size and not significantly different. The cooler temperatures of 5°C and 6°C resulted in significantly smaller lesions than those at all other temperatures. Temperatures from 10°C to 15°C resulted in lesions sizes that are significantly different to each other and all other temperatures.

Genotype had a significant effect on lesion size (Figure 5.14.B, Table 5.13). The mean lesion size of genotypes 6_A1 and 2_A1 were not significantly different from one another. Genotype 6_A1 and 2_A2 had significantly larger lesions than 13_A2 and 17_A2. Genotype 17_A1 had significantly smaller lesions than the other isolates. Genotype 13_A2 had an intermediate lesion size and was significantly different from all the genotypes (Fig 5.20.B).

A significant genotype-temperature interaction was seen (P <.001). At 5°C, genotype 13_A2 and 17_A2 did not grow, genotype 2_A1 had the largest lesions (with an average of 0.94 mm^2) and 6_A1 had a lesion size of 0.09 mm^2; none of the genotypes were significantly different from one another at this temperature, it was not until a temperature of 7°C that significant differences were noted. As the temperature increased, the differences between the size of the lesions formed by each genotype were more obvious. Genotype 17_A2 formed the smallest lesions out of the four genotypes in this test. At every temperature 17_A2 formed the smallest lesions and at a mean of 17°C the lesions were markedly smaller than at 16°C. This marked drop in lesion size

was not seen in other genotypes. Genotype 2_A1 and 6_A1 were not significantly different to one another at any of the mean temperatures but they both had significantly larger lesion sizes than genotype 13_A2 and 17_A2. Genotype 13_A2 had an intermediate lesion size throughout; at temperatures below 9°C and 12°C it was not significantly different from genotype 6_A1 and 2_A1, but at all other temperatures it had significantly smaller lesions.

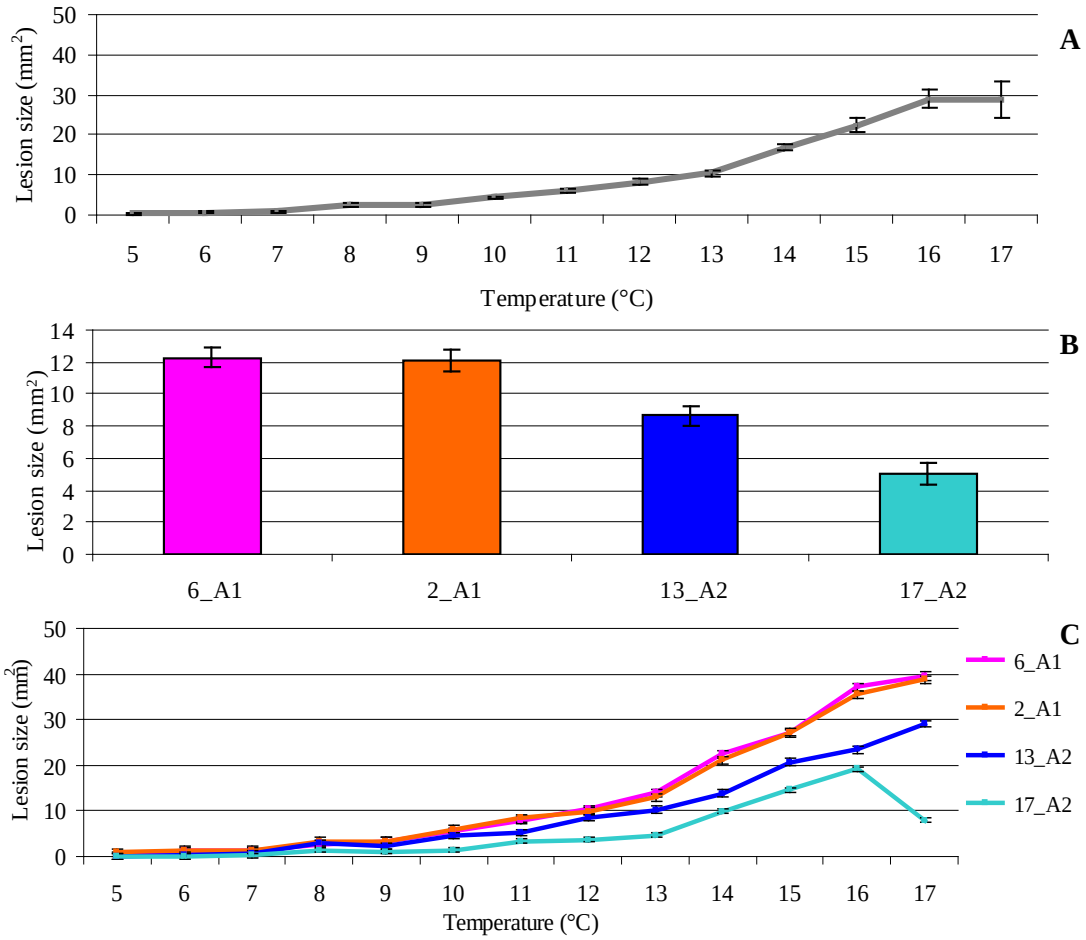


Figure 5.14 – Mean lesion size (mm^2) for four UK *P. infestans* genotypes on Maris Piper detached leaflets that were incubated on a temperature gradient plate to provide different day and night temperature patterns for each point on the plate. Each temperature combinations has been meaned. . The errors bars represent the standard errors of differences of the means (SE)

A) Mean lesion size for each temperatures meaned over genotype. SE: 5°C 0.211, 6°C 0.233, 7°C 0.259, 8°C 0.343, 9°C 0.437, 10°C 0.474, 11°C 0.551, 12°C 0.765, 13°C 0.692, 14°C 0.893, 15°C 1.706, 16°C 2.282 and 17°C 4.523

B) Mean lesion size for each genotype meaned over temperature. SE: 0.649

C) Mean lesion size for each genotype at all meaned temperature combinations. SE: 13_A2 0.705, 17_A2 0.506, 2_A1 0.809 and 6_A1 0.865

5.3.4.3.1 Day and night combinations

A significant effect of day and night temperatures on lesion size was observed (P <.001, Figure 5.15, Table 5.13, Appendix vi). As expected, genotypes incubated at temperature combinations that included higher temperatures produced larger lesions than those incubated at combinations that included lower temperatures. When looking at the lesion size range for all temperature combinations with the same day temperature, those with the higher night temperatures produced the largest lesions; for example for the combination 12°C/8°C the lesion size was 3.04 mm^2 and for the combination 12°C/16°C the lesion size is significantly larger at 14.29 mm^2. The combination that resulted in the largest lesions was 17°C/15°C, although this was not significantly different from the combinations 17°C/16°C, 17°C/13°C and 16°/16°C. Little growth occurred at the lower temperature combinations such as 5°C/5°C, 5°C/7°C, 6°C/8°C, 6°C/13°C and 8°C/5°C with all lesions under 1 mm^2.

Different genotypes had significantly different lesion sizes (P <.001, Figure 5.15, Table 5.13). Below 10°C, 5°C/5°C, 5°C/7°C, 6°C/8°C and 8°C/5°C, there were no differences between the genotypes. At the combination 8°C/8°C 13_A2 formed a large lesion (in relation to the other genotypes at this combination) of 3.15 mm^2; genotype 2_A1 formed a smaller, but not significantly smaller, lesion (1.86mm^2). A clear difference between the response of 13_A2 and 6_A1 was observed as the day/night combinations warmed. At 8°C/15°C and 8°C/16°C for example, there was no difference between the two genotypes but at 10°C/15°C, 10°C/16°C, 12°C/15°C and 12°C/16°C 13_A2 formed markedly smaller lesions than 6_A1. This effect was continued up at the warmer conditions such as 14°C/13°C, 14°C/15°C, and 17°C/12°C to 17°C/16°C. 6_A1 and 2_A1 behaved similarly over these temperature ranges whereas 17_A2 most commonly formed the smallest lesions.

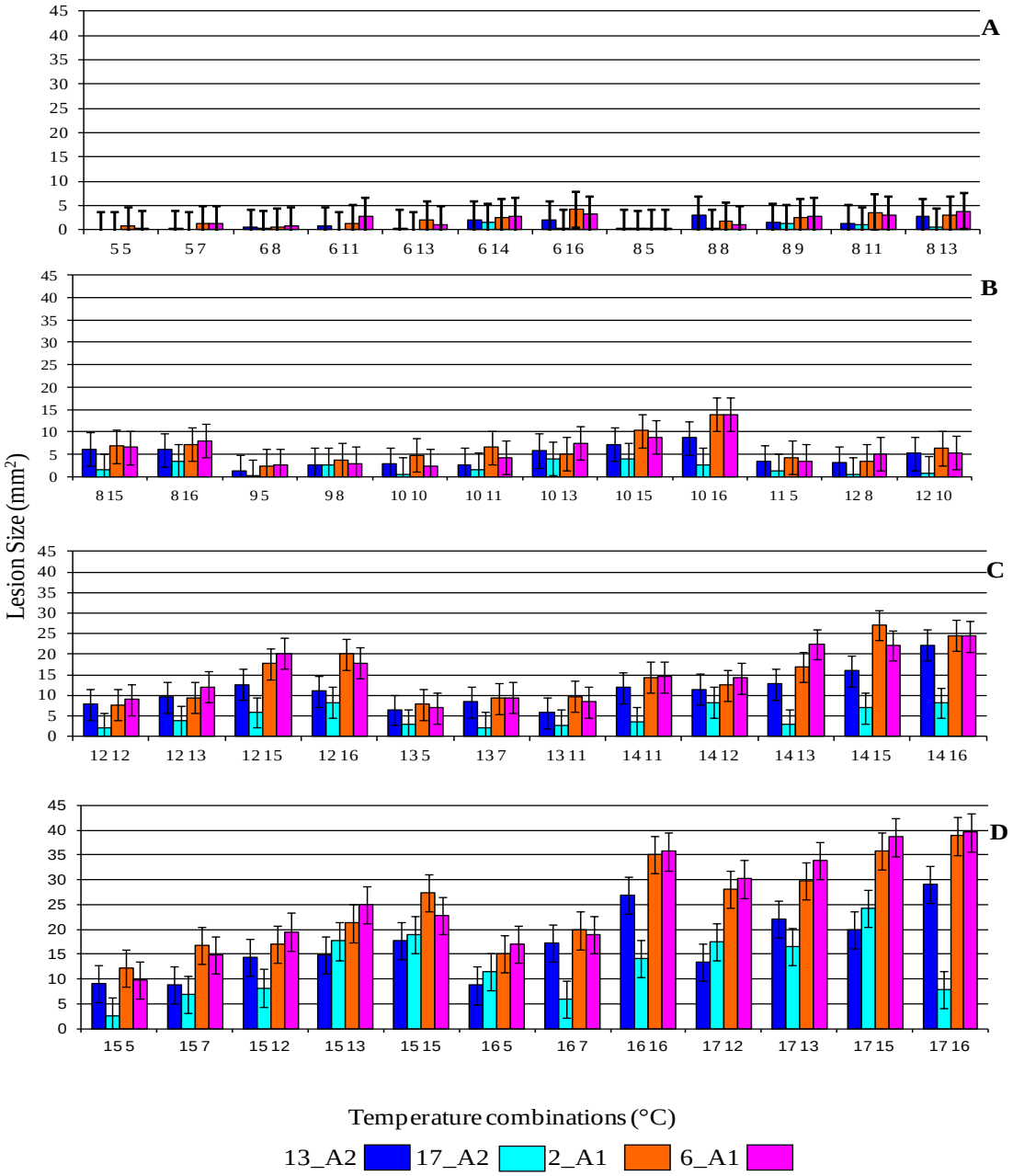


Figure 5.15 – Mean lesion size for four UK *P. infestans* genotypes on Maris Piper detached leaflets that were incubated on a temperature gradient plate to provide different day and night temperature combinations. The errors bars represent the standard errors of differences of the means (SE). SE= 2.17

A) Mean lesion sizes values for temperature combinations ranging from 5°C/5°C to 8°C/13°C

B) Mean lesion sizes values for temperature combinations ranging from 8°C/15°C to 12°C/10°C

C) Mean lesion sizes values for temperature combinations ranging from 12°C/12°C to 14°C/16°C

D) Mean lesion sizes values for temperature combinations ranging from 15°C/5°C to 17°C/16°C

5.3.4.3.2 Accumulated day degrees

A clear relationship was observed between accumulated day degrees and lesion size. Genotype 6_A1 formed the largest lesions at most of the locations on the temperature gradient plate (Figure 5.16). At the warmest locations on the plate, genotype 6_A1 formed the largest lesions (over 30 mm^2). Genotype 2_A1 had three lesions that were above 30 mm^2; all at the warmest locations on the plate. Genotype 17_A2 formed no lesions over 25 mm^2. Genotype 13_A2 generated lesions intermediate in size and did not show the same patterns seen for IP and LP which showed that genotype 13_A2 infected faster than other genotypes at the cool/mid-range temperatures and sporulated faster at the mid-range temperatures.

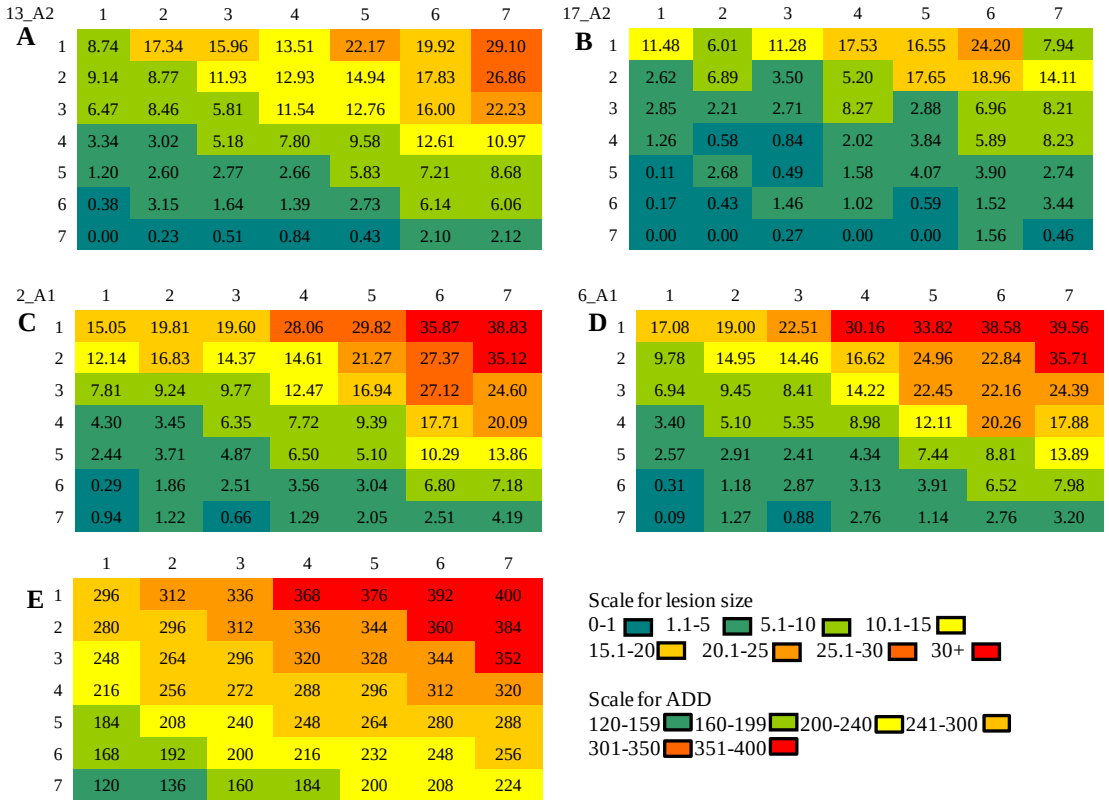


Figure 5.16 – Mean lesion size values for four UK *P. infestans* genotypes on Maris Piper detached leaflets for each point on the temperature gradient plate. Each point represents a different temperature combination.
A) Genotype 13_A2
B) Genotype 17_A2
C) Genotype 2_A1
D) Genotype 6_A1
E) The accumulated day degrees (ADD) for each point on the temperature gradient plate.

Table 5.11 – Descriptive statistics for diurnal *in vivo* temperature investigations; testing how temperature combinations affect IP values of *P. infestans* genotypes on Maris Piper detached leaflets.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
Average temperature	12	385.19	32.09	15.45	<.001
Genotype	3	114.71	38.23	18.41	<.001
Average temperature x genotype	36	48.17	1.33	0.64	0.949
Day/Night combination	47	499.76	10.63	5.06	<.001
Day/Night combination x genotype	141	209.71	1.48	0.71	0.994

Table 5.12 - Descriptive statistics for diurnal *in vivo* temperature investigations; testing how temperature combination affects LP values of *P. infestans* genotypes on Maris Piper detached leaflets.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
Average temperature	12	271.57	22.63	24.39	<.001
Genotype	3	11.67	3.89	4.2	0.006
Average temperature x genotype	36	23.04	0.64	0.69	0.916
Day/Night combination	47	317.83	6.76	7.02	<.001
Day/Night combination x genotype	141	86.24	0.61	0.64	0.999

Table 5.13 – Descriptive statistics for diurnal *in vivo* temperature investigations; testing how temperature combination affects lesion size of *P. infestans* genotypes on Maris Piper detached leaflets.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
Average temperature	12	47546.39	3962.20	96.16	<.001
Genotype	3	6826.72	2275.57	55.23	<.001
Average temperature x genotype	36	4616.10	128.22	3.11	<.001
Day/Night combination	47	53207.69	1132.08	30.11	<.001
Day/Night combination x genotype	141	6876.71	48.77	1.30	0.021

5.4 Discussion

5.4.1 Methodology

This study looked at the response of a large and genetically diverse collection of isolates of *P. infestans* to a range of temperatures. Experiments involving the monitoring of *in vitro* growth of mycelium on agar were compared to *in vivo* aggressiveness criteria on potato leaflets. The challenges of running aggressiveness tests were discussed in Chapter 3.4.1. As in Chapter 3, for the *in vivo* study in this chapter we used one standard isolate in all 8 tests to validate comparisons between tests. Generally, the standard isolate was not significantly different for IP and LS between the tests, only at temperatures 14°C, 16°C and 20°C were differences seen. The data for these temperatures for which differences were seen were not analysed because 8 separate ANOVAs would have had to be conducted and this would increase the risk of type 1 statistical errors. Only one standard isolate was used in the study unlike in the aggressiveness test where three were used. It would have been more advantageous to use more than one standard isolate in the test to make sure that other isolates, not only isolate 2006_3928A, were not significantly different. If more standard isolates were used fewer isolates would have been able to be tested in each run of the tests, increasing the number of tests needed and increasing the chance of any variation between the standard isolate. An alternative to using the standard isolate approach would have been to test all isolates at the same time. This however, would have required each temperature to be tested separately in either eight different walk-in growth chambers (which were not available) or in one (or more) walk-in growth chambers in consecutive tests over a longer time period. A disadvantage of this would have been that different temperatures were tested on leaf material and inoculum prepared on several consecutive dates with the risks of variation introduced between tests. The advantage of the approach taken in this study is that the full temperature response profile of each group

of seven isolates was tested under exactly the same conditions on leaf material harvested at the same time.

More information could have been gained out of this study by testing the isolates on more than one cultivar. Ideally, each test performed on detached leaflets would have been conducted on the same five cultivars used in the aggressiveness test (Maris Piper, King Edward, Estima, Lady Balfour and Cara). Maris Piper was chosen as it is the most widely grown cultivar in the UK. Changing simple aspects of the studies could have provided more information. For example using larger leaflets; however this was, to some extent, restricted by space on the incubator plate. Leaving the tests at the lower temperatures running after the leaflets have been colonised at the higher temperatures would have allowed LP to be measured for the lower temperatures. This would, however have extended each experiment so fewer tests could be run in a single growing season. It could be argued that the lower limits of LP are not so relevant because during a potato growing season it is very unlikely that there will be a prolonged period of a constant temperature below 10°C, but it would have been scientifically interesting to understand such responses.

5.4.2 Within and between-genotype variation

Marked variation was seen amongst isolates of a single genotype in the *in vitro* and *in vivo* experiments in this study. A similar level of within-genotype variation was seen in the study on aggressiveness (Chapter 3) in the IP, LP and lesion size values of some genotypes. Genotypes were categorised by the alleles at each of 12 SSR markers that define a genotype. Isolates with identical or closely matching allele combinations are classed as members of the same clonal lineage (genotype). It might be expected that isolates of the same genotype would behave more similarly to each other than to isolates of other genotypes. However, a level of variation within a genotype has been reported

and may relate to mutations that have accumulated since the lineage was generated. Goodwin (1997) stated that the rate of mutation would not have to be excessively high to account for the observed levels of variation within *P. infestans*. In addition to mutation, epigenetic changes will likely have an impact. Epigenetic changes are heritable modifications to gene expression or phenotype that do not involve changes in the DNA sequence. Gene silencing in relation to the RNA interference pathway and proximity to transposable elements for example, has been reported, in *P. infestans* (Whisson *et al.*, 2008; Vetukuri *et al*., 2011; Whisson *et al*., 2012). A significant amount of variation in colony size, lesion size IP and LP within each genotype was observed in this study. Vast numbers of sporangia are produced from even a single infected field with each one exposed to selection pressures such as host resistance, fungicides and environmental conditions. It is therefore quite plausible that diversity that explains the observed within-genotype variants could be generated.

Isolates of genotype 13_A2 have a common genetic background, but a large amount of variation between the isolates of a genotype was observed. Such large variation amongst the isolates of a single genotype was not seen for all genotypes. In general there was less variation in genotypes that had fewer representative isolates, i.e. genotype 1_A1 or genotype 10_A2. There are two possible explanations for this; the lack of variation is may be due to the small numbers being tested for that one genotype and if more isolates of a genotype were tested then the chance of sampling a greater variation within the genotype may increase. Secondly the differences in population size of the genotypes may be having an effect. The overall population of genotype 13_A2 is much larger than other genotypes with a greater scope for mutation or epigenetic change to have emerged.

In both the *in vitro* and the *in vivo* study, there was more variation seen between the genotypes at the extreme temperatures compared to the optimal temperatures. But there

were few similarities for the genotypes between the tests, for example in the *in vitro* study, genotype 3_A2 had the largest lesion size at most temperatures but this was not translated to the *in vivo* study. In the *in vivo* study, genotype 13_A2 had larger lesions than genotype 6_A1 at both 6°C and 8°C. At the other temperatures both have a similar lesion size. Genotypes did not show any clear patterns that were similar for each temperature as ranking was not consistent for most genotypes for each temperature. The variation amongst the isolates masks the differences between the genotypes.

The variant within genotype 13_A2 showed different levels of aggressiveness; genotype 13_A2_5 was less aggressive than both of the other variants within genotype 13_A2, genotype 13_A2_1 and 13_A2_2 (data not shown). This may explain variation within some genotypes such as genotype 13_A2, but it is not a valid explanation for all genotypes. The collection of isolates used did not contain variants of genotype 6_A1 and more variation was seen within genotype 6_A1. The year of isolation will affect the amount of variation seen within genotypes; as more time passes since the formation of the genotype, more mitotic events occur which leads to variation between isolates, for example, the aggressiveness test in Cooke *et al*., (2012a) only had isolates from the year 2006 so less variation could potentially be seen compared to the isolates used in this study as the year of isolation covered a three year period.

5.4.3 Activity at lower temperatures

The Smith Period states that the minimum temperature for a Smith Period to occur is 10°C (Smith, 1956), although it must be remembered that the Smith Period was defined on the basis of patterns in weather data and how this related to observed disease outbreaks. Smith was not attempting to define biological thresholds. In this study, it has been shown that UK genotypes of *P. infestans* can infect potato at temperatures below 10°C, even as low as 6°C. One set of isolates incubated at 6°C in the *in vivo*

study generated very small hypersensitive response-like lesions that, once incubated at 15°C, rapidly spread to colonise the leaflet completely. This demonstrated that the isolates did indeed form viable lesions at constantly low temperatures even if it does take a long time. This knowledge suggests that predictions based on the Smith Period alone may underestimate the risk of blight activity. Crucially, it is likely that infections early in the season when the nights are cool could be missed. Infection below 10°C was not just limited to genotype 13_A2, some isolates of all genotypes, except 3_A2, could infect at temperatures below 10°C. In the diurnal experiments, genotype 17_A2 performed well at the lower temperatures also. It has to be remembered that the Smith Period was first proposed in the 1950s and since then there is evidence that the populations of *P. infestans* have changed dramatically over the years with the most recent being the dramatic increase in genotype 13_A2.

Generally, growth below 10°C is slow for *P. infestans*. Even when the temperature is fluctuating between low and high temperatures e.g. in the diurnal temperature experiment with 16 hours at 6°C and 8 hours at 12°C, little growth is seen. Out of the 49 isolates used in the *in vivo* study, only 24 isolates infected and usually it was only one of the replicates that became infected for each of the 24 isolates. This shows that infection is very sporadic at this temperature.

Harrison (1992) stated 'Although the Beaumont and Smith developed blight-forecasting systems based on the assumption that there is little fungal development below 10°C, it would be risky to rely on this rather arbitrary cut-off temperature'. It is not the first time that *P. infestans* has been shown to grow at such low temperatures. Hartill *et al*. (1990) studied the effect temperature had on sporangia formation of isolate CG3, which is metalaxyl resistant, at a range of temperatures; 5°C, 8°C, 10°C, 12°C, 13°C, 14°C, 15°C, 18°C, 20°C and 22°C. At 5°C, Hartill *et al*, (1990) found that the LP was 12 days. Sporulation below 8°C takes a long time and that duration of constant low

temperature during a potato season is unlikely. It is important to know that pathogen development still occurs in the crop during the cooler night time temperatures even as low as 5-6°C. Even small differences in the ability to grow or the amount of growth under such conditions will affect the disease development as it provides an opportunity for infection under marginal conditions when the correct temperature criteria and moisture availability may only occur together for short period of time. If infection occurs under these conditions the pathogen may then continue to develop and establish a lesion within the leaf as the temperature rises during the daytime. In polycyclic diseases such as late blight apparently minor differences in IP and LS between the isolates may equate to larger differences in field epidemic development because the effect will be cumulative for each progressive life cycle. It may be better to suggest that the Smith Period should be about detecting growth at below 10°C, but saying there would only be sporulation at above 10°C.

Sporulation is affected by temperature and humidity. At low temperatures, in order for sporulation to occur there need to be longer hours of high humidity compared to the amount of high humidity needed at optimal temperatures. Rotem *et al.* (1970) investigated the effect of temperature on infection in conjunction with inoculum load and leaf wetness. Six temperatures ranging from 5-28°C were investigated along with five spore concentrations and four leaf wetness durations. No minimum temperature for infection, within the range tested by Rotem *et al*. (1970), was found when the leaf wetness duration was 12 or 24 hours. A minimum temperature of 10°C was seen at 6 hours of wetness regardless of inoculum concentration. In the study reported in this thesis the humidity was presumed to be maintained at 100% as all leaflets were contained in Petri dishes that were lined with moist filter paper; it could be postulated that the leaf wetness or humidity on a rainy day would be high enough within the crop for 12 hours for infection to occur at a low temperatures.

The diurnal study shows an isolate specific temperature effect (Appendix v). Only one isolate is representing the genotype so it cannot be generalised. However, it does show that within the same experiment an isolate of different genotype shows a different response to different temperature combinations. It clearly shows that genotype 13_A2 and 6_A1 react very differently to the temperature combinations with genotype 6_A1 producing a significantly larger lesion than genotype 13_A2 at several different temperature combinations all of which are above 10°C and most of which have a high night temperature. At similar high night temperatures and an 8°C day temperature the difference between genotype 13_A2 and 6_A1 is not seen, showing that genotype 6_A1 is able to cause more disease than genotype 13_A2 at the higher temperatures, even if those higher temperatures are only for a few hours. Although genotype 13_A2 did not cause the most disease compared to genotype 6_A1, between the low and mid-range temperatures, it did infect faster. This could signify that within the field being able to establish an infection earlier on is more important that being able to cause more disease when the temperatures are warmer. Interestingly, in the diurnal study, genotype 17_A2 is the least aggressive genotype even though it performed well in the *in vitro* study, had short IP values at most temperatures in the *in vivo* study and was shown to be aggressive by Cooke *et al*. (2012a).

5.4.4 Genotype 13_A2

Cooke *et al* (2012a) showed that genotype 13_A2 was amongst the most aggressive genotypes of *P. infestans* with the shortest LP values and the largest lesion size when compared to other UK genotypes at 13°C. In the *in vivo* study, no significant effect of genotype was found on the lesion size values at 8°C but genotype 13_A2 still produced the largest lesions. No variation between genotypes was seen for LP values in the diurnal experiment for the lower temperatures, in fact sporulation was sporadic with most temperature combinations showing infection efficiency is lower at cooler

temperatures. In the diurnal experiment, genotype 13_A2 had shorter IP values when the temperature combinations contained a low temperature and when the temperature combination averaged to a temperature between 10°C and 13°C. Mitzubuti and Fry (1998) commented that the small variations seen in the optimum temperatures can have large effects on field epidemics. Genotypes that can infect at lower temperatures would establish an epidemic faster due to being more active during the cooler night temperatures. It may be the case that genotype 13_A2 is dominant not because it is the fastest coloniser of the crop but that its low level infection goes undetected in the crop before fungicides are sprayed, effectively giving this genotype an advantage in establishing an epidemic even before conditions are optimal.

Although all genotypes have the capability to infect at the lower temperatures, as Rotem *et al*. (1970) showed, the limits of biological parameters are relative to other biological parameters, so it is far too simplistic to state one genotype dominates due to one reason alone. Dominance in the population may depend on a great number of factors other than the specific traits measured in this study, for example, fungicide resistance, the amount of primary inoculum available at the start of next growing season and the weather during that season. For example, in 2011 genotype 6_A1 dominated the UK *P. infestans* populations (D. Cooke personal communication). It was suggested that this is due to the epidemic starting in the warmer conditions in late July compared to earlier in most recent seasons. Also much of the primary inoculum was likely to have failed as a result of the very dry spring. This study showed that genotype 6_A1 is better adapted to higher temperatures than genotype 13_A2. In the diurnal experiment, genotype 6_A1 was shown to be more effective at infecting and colonising the leaflet at the warmer temperatures so although genotype 13_A2 has a broader virulence profile and infected leaflets slightly faster at the cooler temperatures, genotype 6_A1 may have dominated

the UK population in 2011 because of the selection pressures applied by the environment.

5.4.5 Conclusion

There was no conclusive evidence to show that genotype 13_A2 outperformed other genotypes at lower temperatures. There are many characteristics necessary to be a successful pathogen and under the conditions in this study we were not able to identify any clear preference or adaptation to temperature. It would appear that a broader picture needs to be examined in order to get the clearest indication of why genotype 13_A2 was the fittest genotype of *P. infestans* in UK from 2005-2010. Infection below 10°C was seen within the *in vivo* study so this does suggest that the Smith Period needs to be modified.

Chapter 6 – The effect of humidity on infection of potato by four *P. infestans* genotypes

6.1 Introduction

Humidity is extremely hard to control experimentally because the area close to the leaflet will increase in humidity as the loss of water vapour via transpiration occurs and the air will be more saturated than the ambient humidity (Harrison, 1992). Air speed affects the amount of water vapour at the leaf surface as it removes the saturated air close to the leaf thus lowering the humidity close to the infection point. Harrison (1992) stated that until an instrument is created that can accurately record the humidity at the leaf surface then only the ambient humidity is measured and the humidity at the infection point can only be guessed. Minogue and Fry (1981), Harrison and Lowe (1989) and Butler *et al*. (1995) designed chambers that controlled the ambient humidity. All designs used a sealed clear chamber to house the plants. Butler *et al*. (1995) used a mixture of moist and dry air to reach the desired humidity, whereas Minogue and Fry (1981) and Harrison and Lowe (1989) reached the desired humidity by passing the air through water to saturate it. The method Harrison and Lowe (1989) used could obtain a relative humidity (RH) within 2% either side of the desired value (Butler *et al*., 1995). The most relevant place to measure humidity is at the microclimate next to the infection point but the act of measuring the conditions of the leaf surface could alter the humidity itself (Harrison, 1992).

6.1.1 Humidity and the Smith Period

Potato late blight epidemics depend on the presence of inoculum and particular climatic factors. Forecasting systems are used by potato growers to predict when there is a potential blight risk, so understanding the biological limits needed for *P. infestans* to infect and sporulate is crucial when creating a potato late blight forecasting system that is accurate and up to date. In the UK the Smith Period is used. The Smith period is

very similar to what Beaumont (1947) suggested as a forecasting system. Beaumont (1947) studied the meteorological data from 1929 to 1939 and developed a 'temperature-humidity' rule stating that the temperature must be 10°C or above and the RH above 75% over two consecutive days. Implementation of this rule provided good protection against blight in Devon and West Cornwall. Smith (1956) studied blight outbreak maps and weather data between 1950 and 1954 and found that having the humidity rule set at a particular duration of high humidity rather than a level it should not fall below reduced the number of false predictions that state there is no blight risk when there was. A Smith Period is defined as two consecutive days where the minimum temperature is 10°C or above and on both days the RH must be 90% or above for at least eleven hours (Smith, 1956).

6.1.2 Aim

Contemporary UK *P. infestans* populations are different from the population that the Smith Period was based upon and questions have been raised about its accuracy. The aim of this study is to assess how many hours at high humidity are needed for isolates of four *P. infestans* genotypes to infect Maris Piper foliage. Maris Piper plants were used in this study as it is the most widely grown cultivar in the UK and has a foliar blight resistance rating of 4 (resistance ratings described in Chapter 1.6.1). Isolates representing the four genotypes used in this study (13_A2, 6_A1, 2_A1 and 17_A2) are the same as those used in Chapter 5.2.3. To monitor the growth of the *P. infestans* genotypes incubation period (IP) was monitored and percentage foliar blight scores were recorded 10 days post inoculation. The experiment was conducted at three different temperatures (8°C, 10°C and 15°C).

6.2. Method

In vivo growth of *P. infestans* was tested in a single stem plant assay to investigate how many hours of high humidity were needed for *P. infestans* to infect and grow. Four isolates were used to represent the four genotypes present in the study; 2006_3928A (genotype 13_A2), 2008_6090A (genotype 6_A1), 2007_5442F (genotype 2_A1) and 2006_4388D (genotype 17_A2). The fifth treatment was a water control. This experiment was conducted at three temperatures; 8°C, 10°C and 15°C. Isolates were passaged through the potato cultivar Craig's Royal (susceptible) twice before being used (as described in Chapter 2.4). Tubers of cv Maris Piper were planted 4 weeks prior to the experiment in a temperature controlled glasshouse which was set at 18°C and had 16 hours of natural light regime supplemented with artificial light.

6.2.1 Design

Fifteen clear plastic boxes (65 x 41 x 59 cm) were lined with tissue paper and 600 ml of water was used to dampen the tissue. A beaker containing 300 ml of water was placed in the corner of each box so that humidity would remain high throughout the experiment. To record the humidity within the boxes humidity loggers (DS1923 Hygrochron iButton, Maxim Direct UK) were used and these were placed on top of inverted 100 ml beakers to keep them off the damp tissue. Ten single-stem whole plants, that were four weeks old, were placed into each box and a sealable lid was attached to maintain humidity. Within the boxes, each plant was randomly assigned to a humidity period after which it was taken out of the box; the position of each plant representing a specific humidity period was randomised and the boxes were randomised within replicates. A fully randomised block design was used with three replicate blocks containing 5 treatments (4 isolates and a water control).

6.2.2 Inoculation and incubation

Seven day old sporulating lesions on detached Craig's Royal leaflets were used to make the sporangial suspension for each isolate (as described in Chapter 2.4). Each box within the replicates was assigned one treatment and the ten single stem whole plants within the box were spray inoculated with the sporangial suspension of that treatment. The boxes were covered in black plastic sheeting and incubated in the dark in a walk-in growth chamber at a set temperature, 8°C, 10°C or 15°C.

6.2.3 Data collection and analysis

At specific humidity periods (0, 1, 2, 4, 6, 8, 10, 14, 18, and 24 hours post inoculation) one plant from each box was removed and placed into a temperature controlled glasshouse set to 15°C. Before the boxes were opened to remove the plant, misters were turned on in the growth room. This meant that only saturated air would be present in the chamber whilst the lids of the boxes were off to prevent any loss of humidity within the box. The single stem plants were monitored by eye for 10 days and IP and percentage foliar blight were scored (Chapter 2.5). The statistical analysis method used was described in Chapter 2.8.

6.3 Results

Originally, this study included isolate 2008_6090A (genotype 6_A1) but it did not infect any of the whole plants at any of the temperatures so it was removed from the analysis. On average the humidity was approximately 98% in all boxes and at all temperatures. The range of humidity in the 15 boxes at 8°C was 96%–100%, for 10°C 93%–100% and for 15°C 93%–100% An ANOVA was conducted between the replicates at each temperature to see if variation could be due to the position with the growth chamber. For IP and percentage foliar blight, there were no significant differences between the replicates; $P = 0.939$ and $P = 0.895$. No sporulation was seen on any plant so LP has not been included in the analysis. The water controls showed no sign of infection or foliar blight; these data were not included in the analysis. Analysis included the non-infecting treatments if other treatments of the same isolates had infected as these represent true results rather than a failure of the inoculum.

6.3.1 Incubation period

All plants that were not infected were assigned an arbitrary IP of 11 days as this is the length of the experiment plus one day.

6.3.1.1 Humidity period

The number of hours at high humidity that the plants were incubated for during infection had a significant effect on the IP values ($P<0.001$, Figure 6.1.A, Table 6.1). Plants sampled immediately after inoculation and those sampled after 1 hour at high humidity had an IP value of 11.0 days showing that no infection had occurred. After 2 and 4 hours at high humidity the IP values were significantly longer than the IP values after 6 or more hours of high humidity. IP values after 6 and 8 hours of high humidity were significantly different from IP values at all other humidity periods and significantly different from each other. After 10 or more hours of high humidity the IP

values were not significantly different from one another but had significantly shorter IP values than humidity periods under 10 hours.

6.3.1.2 Isolate

There were significant differences in IP values between the isolates (P<0.001, Figure 6.1.B, Table 6.1). Isolate 2007_5442F (genotype 2_A1) infected faster than the isolates 2006_3928A (genotype 13_A2) and 2006_4388D (genotype 17_A2) after most periods of high humidity and this difference was significant for tests at 2, 4 and 8 hours of high humidity. After 10 or more hours of high humidity, isolates 2006_3928A and 2007_5442F did not significantly differ from one another but had significantly shorter IP values than 2006_4388D.

6.3.1.3 Temperature

Temperature had a significant effect on the IP of the isolates (P<0.001, Figure 6.1.C, Table 6.1). At 15°C, all mean IP values were longer than at 8°C and 10°C. In some cases this difference was significant, for example, there were significant differences in the IP values for the isolates 2006_4388D and 2007_5442F, whereas the difference was not significant for isolate 2006_3928A (Figure 6.1.C). The mean IP values at 8°C for isolates 2006_3928A and 2006_4388D were not significantly different from one another, but at 15°C isolate 2006_4388D had a significantly longer IP.

6.3.1.4 Infection rate

After 10 or more hours of high humidity the majority (93%) of the treatments had infected. After 8 hours at high humidity 85% of the treatments had infected. With 6 hours of high humidity the percentage infection rate was less than 33%, dropping to 4% after only 1 hour's high humidity. The longer IP values were produced because of the averaging of those treatments that did not infect and treatments that did infect. Treatments that did infect after 8 or fewer hours of high humidity had lower IP values

on most occasions than treatments that infected at 10 or more hours of high humidity, but were in the range of 4–11 days.

Table 6.1 – Descriptive statistics for humidity investigations: testing how the combination of hours at high humidity and the temperature at which Maris Piper whole plants were incubated at inoculation affected the IP of three *Phytophthora infestans* isolates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
Hour	9	1821.9	202.9	150.2	<.001
Isolate	2	189.8	94.9	70.4	<.001
Temperature	2	82.0	41.0	30.4	<.001
Hour x Isolate	18	120.8	6.7	5.0	<.001
Hour x Temperature	18	73.9	4.1	3.1	<.001
Isolate x Temperature	4	21.3	5.3	4.0	0.004
Hour x Isolate x Temperature	36	126.3	3.5	2.6	<.001

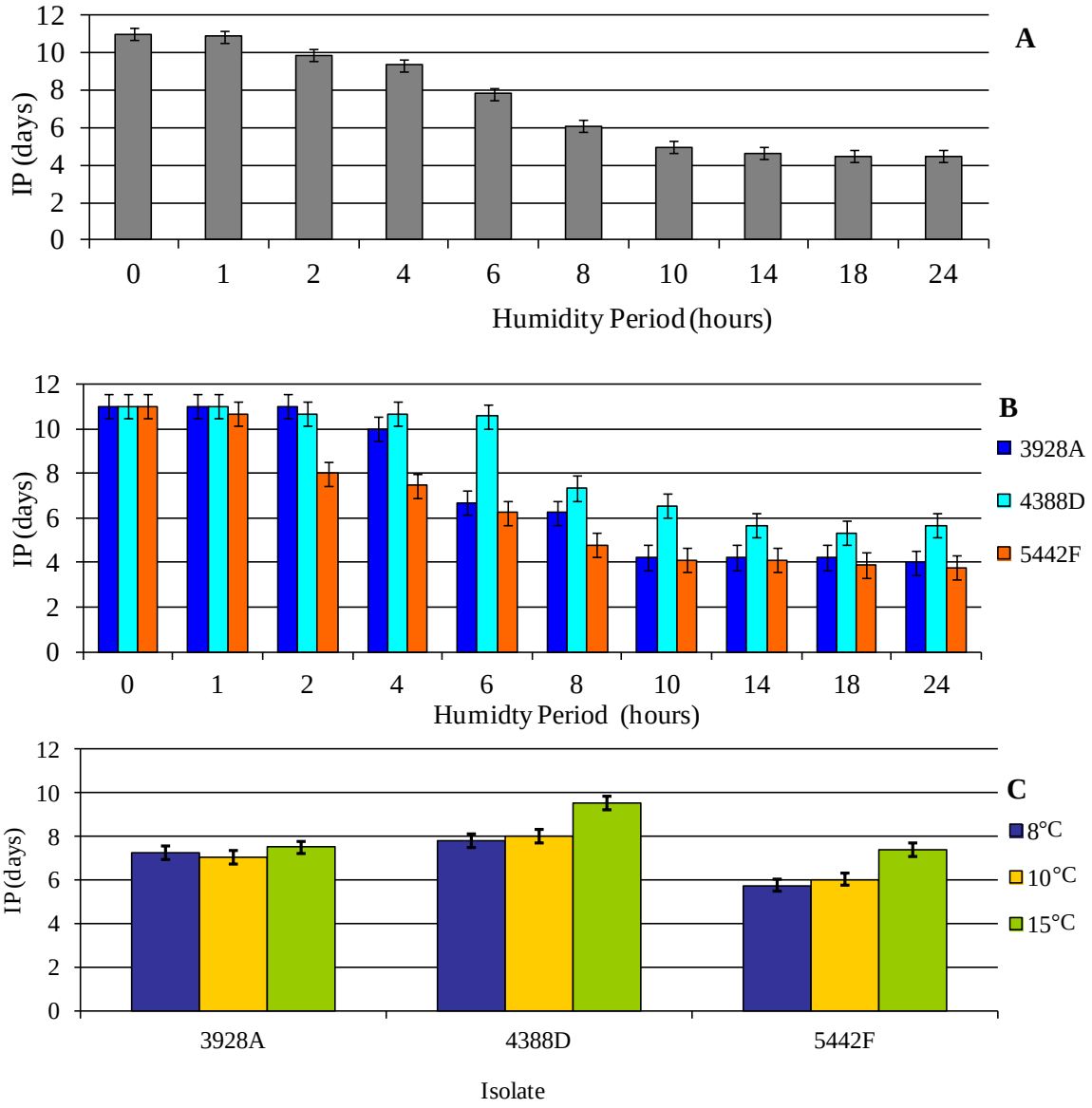


Figure 6.1 – Effect of different periods of high humidity at three temperatures on infection of Maris Piper plants by three isolates of *Phytophthora infestans*.

A) Mean IP values meaned over treatments for each temperature SE= 0.316

B) Mean IP values for the four treatments at each time point SE= 0.547

C) Mean IP values for the four treatment at the different temperatures meaned over all time points SE= 0.299

6.3.1.5 Interactions between humidity, temperature and isolate

Significant interactions for humidity period, temperature and isolate were seen (Table 6.1, $P<0.001$). For isolate 2006_3928A, there was no significant effect of temperature on the IP values after 8 or more hours of high humidity. After 6 hours at high humidity, isolate 2006_3928A had a significantly shorter IP at 10°C with a mean IP value of 4.0 days compared with 8°C and 15°C which both had a mean IP value of 8.0 days (Figure 6.2.A). Whereas, at 4 hours of high humidity the IP for treatment at 8°C was significantly shorter (8.0 days) than those of the other two temperatures (Figure 6.2.A). No infection was recorded after 2 hours of high humidity or fewer. At 8 or more hours of high humidity, isolate 2006_4388D was the slowest isolate to infect especially at 15°C. There was little difference between the mean IP values at the lower temperatures of 8°C and 10°C (Figure 6.2.B). At 0, 1, 2, 4 and 6 hours of high humidity all mean IP values were not significantly different. Isolate 2007_5224F was by far the fastest isolate to infect the Maris Piper whole plants (Figure 6.2.C). Temperature had no significant effect on the IP values after 8 or more hours of high humidity. After 2 to 6 hours of high humidity, the IP value at 15°C was significantly longer than the IP values at the other temperatures. After 2 hours of high humidity, all mean IP values for each temperature were significantly different, with infection at 8°C taking the shortest time (5.0 days).

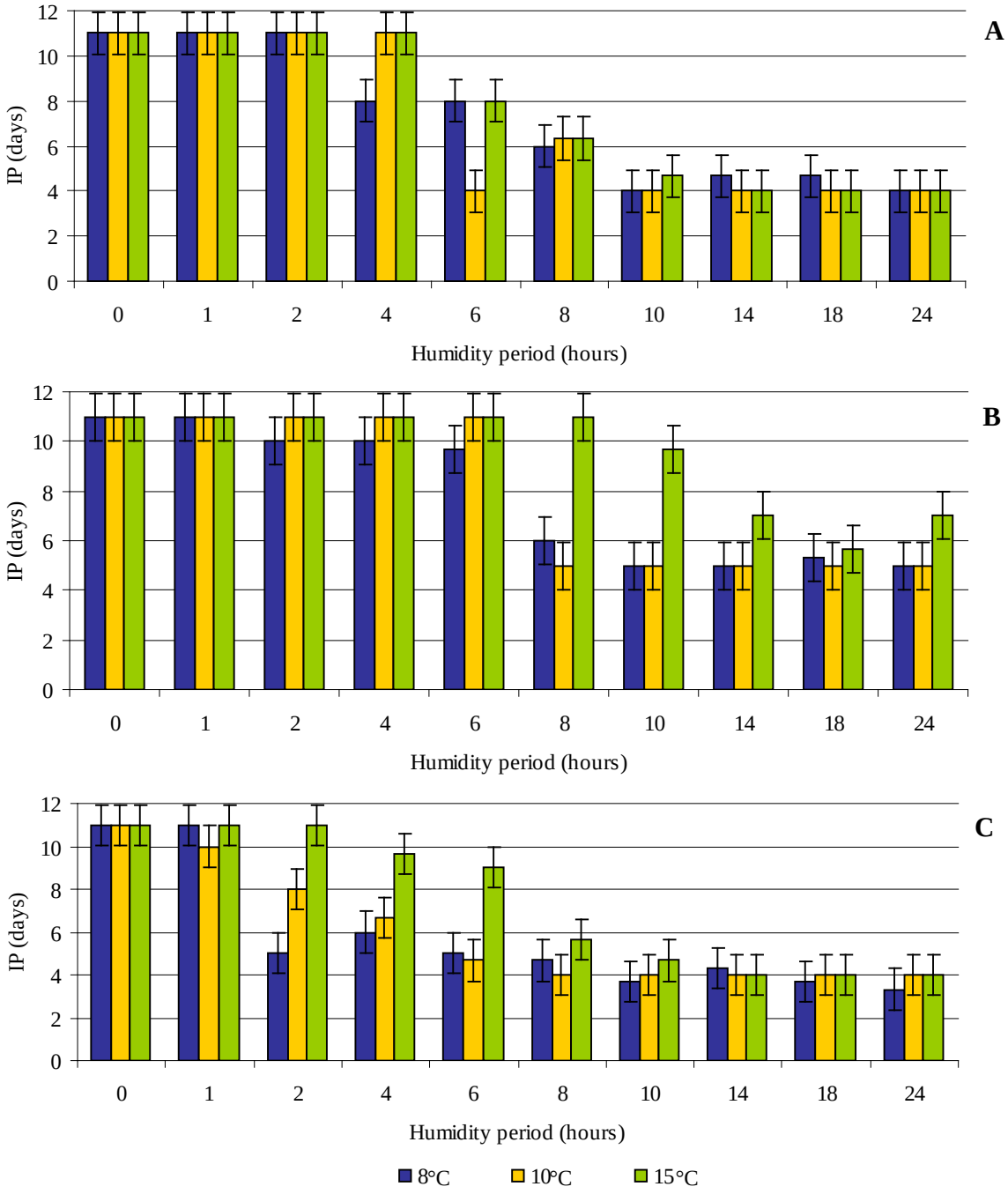


Figure 6.2 – Effect of different period of high humidity at three temperatures on infection of Maris Piper whole plants by three isolates of *Phytophthora infestans*. SE=0.948

A) 2006_3928A B)2006_4388D C)2007_5442F

6.3.2 Foliar blight

6.3.2.1 Humidity period

The number of hours at high humidity had a significant effect upon percentage foliar blight (P<0.001, Table 6.2). There was no difference in foliar blight after 18 hours of high humidity and 24 hours of high humidity (Figure. 6.3.A). The percentage foliar blight after 10 hours of high humidity was 34.7%, significantly less than the percentage foliar blight present on plants after 14, 18 and 24 hours of high humidity but significantly greater than the percentage foliar blight on plants incubated for 8 hours or fewer at high humidity. There was foliar blight present on those plants incubated for 2 hours of high humidity, although it had a very low mean percentage of 1.5% (Figure 6.3.A).

6.3.2.2 Isolate

Isolate had a significant effect on the foliar blight present on the Maris Piper single stem plants (P<0.001, Table 6.2). Plants infected with isolate 2007_5442F (genotype 2_A1) had a significantly greater percentage foliar blight present, with a mean value of 45.0% (Figure 6.3.B). Isolate 2006_3928A (genotype 13_A2) produced significantly less disease compared to isolate 2007_5442F, but significantly more disease than isolate 4388D (genotype 17_A2) (Figure 6.3.B).

6.3.2.3 Temperature

Temperature had a significant effect on the amount of foliar blight present on the plants (P<0.001, Table 6.2). Plants incubated at 10°C during the humidity period had significantly more foliar blight compared with plants incubated at 8°C and 15°C. At 15°C, the development of symptoms occurred more slowly than other temperatures.

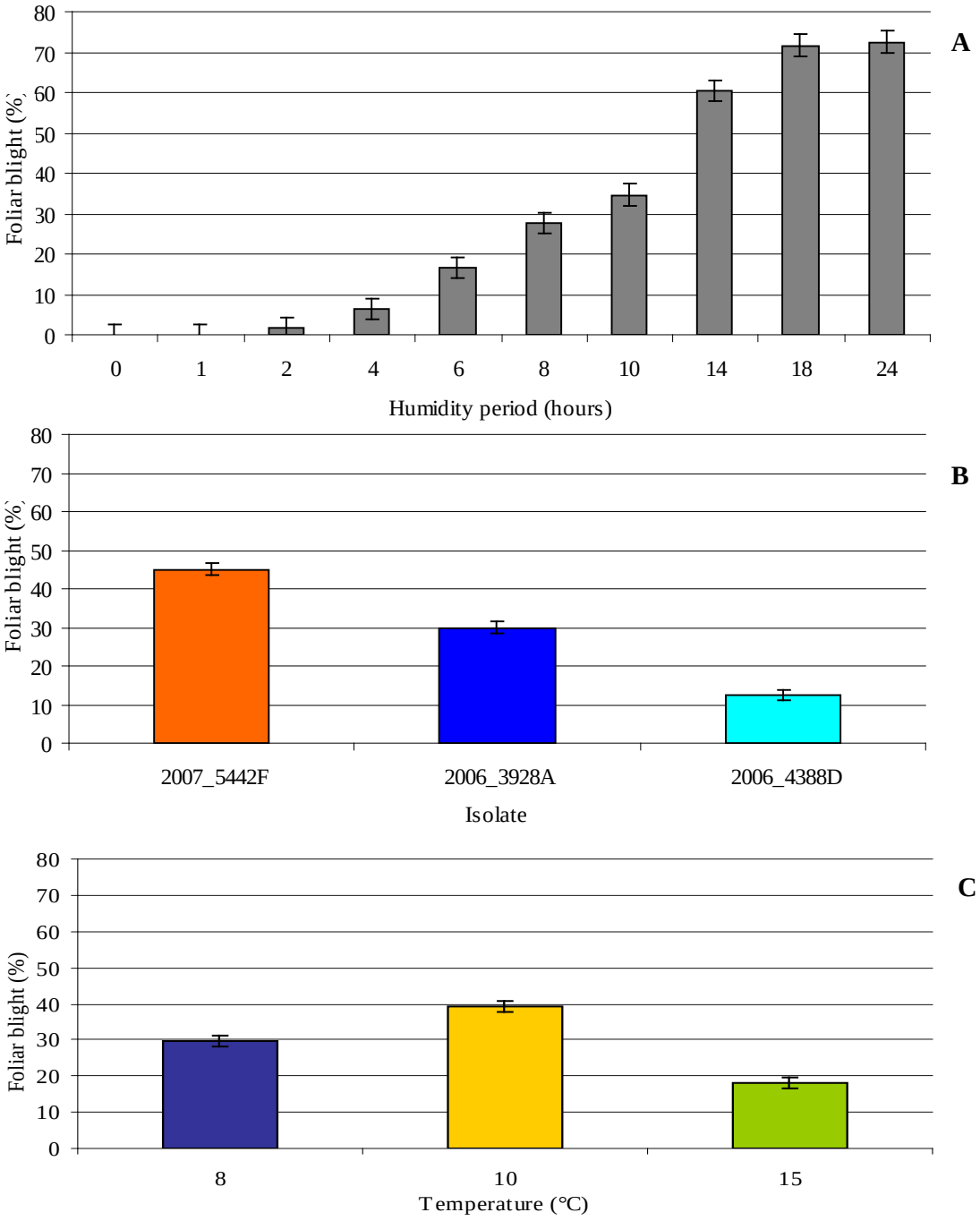


Figure 6.3 – Effect of different period of high humidity at three temperatures on percentage foliar blight of Maris Piper whole plants by three isolates of *Phytophthora infestans*.

A) Mean percentage foliar blight for each time point (meaned over all treatments and temperatures). SE=2.673

B) Mean percentage foliar blight for each treatment (meaned over all times points and temperature). SE=1.464

C) Mean percentage foliar blight for each temperature (meaned over all time points and treatments). SE=1.464

6.3.2.4 Interactions between humidity period, temperature and isolate

Significant interactions for all three factors (humidity period, temperature and isolate) were seen for percentage foliar blight ($P<0.001$, Table 6.2). Maris Piper plants infected with isolate 2006_3928A (genotype 13_A2) had a small percentage foliar blight after 4 hours of high humidity when incubated at 8°C during infection but did not develop any foliar blight at the other temperatures (Figure 6.4.A). In most cases, more foliar blight was seen on the plants incubated at 8°C during the humidity period than at the other temperatures tested (Figure 6.4.A). Isolate 2006_4388D (genotype 17_A2) produced the least foliar blight. At 15°C, it caused very little foliar blight, ranging from 0.3% to 1.0% after 10 to 24 hours of high humidity. Foliar blight developed after 4 and 6 hours of high humidity only at 8°C. Isolate 2006_4388D required a long period of high humidity to cause large amounts of foliar blight e.g. after 24 hours at high humidity at 8°C it produced 80% foliar blight. Isolate 2007_5224F (genotype 2_A1) was the most aggressive causing 100% foliar blight after 8 hours at high humidity. Symptoms developed faster when plants were incubated at 10°C during the humidity period than those incubated at 8°C or 15°C. At both 8°C and 15°C foliar blight only reached 90% after 18 hours of high humidity.

Table 6.2 – Descriptive statistics for humidity investigations: testing how the combination of hours at high humidity and the temperature at which Maris Piper whole plants were incubated at inoculation affected the percentage foliar blight of three *Phytophthora infestans* isolates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Variance Ratio	P Value
Hour	9	211412.2	23490.2	243.4	<.001
Isolate	2	47936.2	23968.1	248.4	<.001
Temperature	2	20062.7	10031.3	104.0	<.001
Hour x Isolate	18	32199.6	1788.9	18.5	<.001
Hour x Temperature	18	15374.1	854.1	8.9	<.001
Isolate x Temperature	4	6600.6	1650.1	17.1	<.001
Hour x Isolate x Temperature	36	20071.1	557.5	5.8	<.001

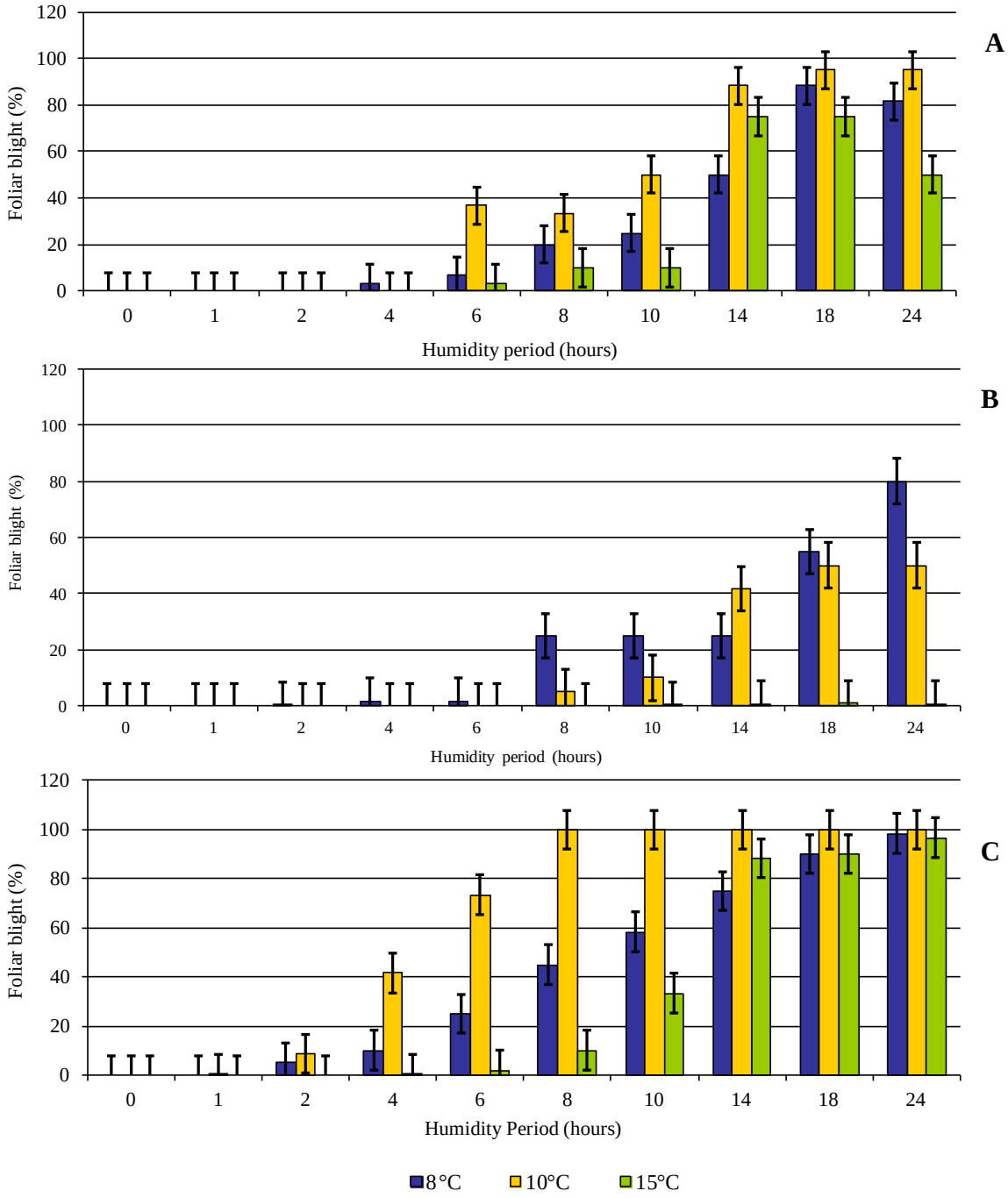


Figure 6.4 – Effect of different periods of high humidity at three temperatures on percentage foliar blight of Maris Piper whole plants by three isolates of *Phytophthora infestans*. SE=8.02

A) 2006_3928A B) 2006_4388D C) 2007_5442F

6.4 Discussion

6.4.1 Controlling humidity

As stated in the introduction (Chapter 6.1), humidity is extremely difficult to control. The method used in this experiment was a rudimentary way of maintaining high humidity but nevertheless it still proved to be effective in creating the high humidity needed for this study. The loggers could only record the humidity around the plant and not directly at the infection point so the microclimate cannot be determined. However, the plants were in a closed box with no air flow so the humidity gradient from the leaf surface to the ambient air would be reduced. The experiment would have been more accurate in testing Smith Period if the RH could have been maintained at 90% as this is the threshold that Smith (1956) states. Maintaining a RH of 90% would have been impossible using the method in this study. In order to maintain 90% RH closed chambers with accurate ways of controlling humidity would have been needed as those used in the study by Harrison and Lowe (1989).

6.4.2 Sporangial survival rate

The sporangial survival rate could have affected this study as some inoculated plants had fewer than four hours in the dark. Sunseri *et al*. (2002) showed that most infections occurred after four hours in the dark with very few infections happening with fewer than four hours of darkness. This was not true for this experiment as infection was seen at the 2 hour time point albeit it from a very small proportion of the isolates.

6.4.3 How does this affect the Smith Period?

It is widely accepted that *P. infestans* needs high humidity to infect but the number of hours needed at high humidity varies between studies. Smith (1956) suggested a cut-off point of 11 hours, Grünwald and Flier (2005) stated 12 to 16 hours at 90% were needed and in fact, most studies maintain high humidity throughout experiments without setting

a standard number of hours at high humidity to ensure infection (Rotem *et al*., 1970; Minogue and Fry 1981; Vleeshouwers *et al* 1999; Turkensteen *et al*., 2000; Carlisle *et al*., 2002; Runno-Paurson *et al*., 2009). Knowing the biological parameters of a pathogen is crucial when creating an accurate forecasting system. In contrast to the RH criterion stated in the Smith Period (as discussion in Chapter 6.1.2), the data in this study showed that the Smith Period needs to be re-evaluated for the contemporary populations as *P. infestans* isolates were infecting after only 2 hours at high humidity. Forecasting based on the Beaumont Period (Beaumont, 1947) was started in England in Wales in the 1950s (Large, 1953). This was subsequently replaced by the Smith Period (Smith, 1956), which was first implemented in 1975 (Bouma, 2007) and it is still used today by UK farmers through systems such as 'Fight Against Blight' and 'Blight Watch' (Hardwick, 2006; Bouma, 2007). Since 1975, there have been two major population changes (as discussed in Chapter 1.8 and Chapter 1.9) and both have led to displacement of the previous population. Novel biological parameters mean that genotypes can become dominant when selection pressures favour them thus giving genotypes an advantage. The Beaumont and Smith Periods related patterns of blight outbreaks to weather conditions rather than being based on biological parameters and have been used for such a long time, not because there were no other better methods but because they gave adequate management of blight risks without the need for any drastic change to the rules (Smith and Walker, 1966). Smith and Walker (1966) suggested that the Beaumont period was used for such a long time because the method was simple to use with the weather data the growers had access to and the limiting factor when collecting data was RH as it was so hard to get accurate hourly data from stations and this could have led to failures in reporting blight risk. It was suggested that the duration of leaf wetness should be examined by measuring the amount of rainfall between 9am and 9pm and 9pm and 9am. If rainfall was present on at least four occasions in a row

with a minimum temperature of 10°C during those time there would be a risk of blight. This would make it easier for the growers to assess blight risk as the equipment needed would be easy to use and inexpensive; all that would be needed is a thermometer and a rain-gauge. This method offered the same level of predicting blight risk as the Beaumont Period (Smith and Walker, 1966). The UK Meteorological Office now has over 200 weather stations across the UK which are used to calculate Smith Periods so accessing Smith Periods is much more accessible now to growers (Barrie and Bradshaw, 2002), and a small change to the rule could be implemented easily.

6.4.3.1 Maximum number of high humidity hours needed for infection and diseases

A maximum amount of hours of high humidity, beyond which infection is not further enhanced, was deduced from this study. For both 2006_3928A and 2007_5442F after 18 and 24 hours at high humidity there was no significant difference between the foliar blight present on the plant. This suggested that after 18 hours at high humidity most of the sporangia had infected and the amount of blight present for that isolate after 10 days had reached a maximum.

6.4.3.2 Minimum number of high humidity hours needed for infection and disease

As stated before, some infection was seen after 2 hours of high humidity but there was not a large amount of disease present on the Maris Piper plants in this treatment. Forecasting systems need to be accurate but also cost-effective, so the small amount of infection after 2 hours of high humidity would not warrant a fungicide treatment, particularly as it must be remembered that the sporangial concentrations used in this experiment were much greater than would be in the natural environment so there would be even less disease seen in a field crop. From the data, lowering the Smith Period humidity criterion to 6 hours would be a better option; much less disease is seen on the Maris Piper plants after 6 hours of high humidity compared to 18 and 24 hours. The

amount of disease present after 6 hours of high humidity was above 10% and was similar to that after 8 hours of high humidity. Cao *et al*. (1997) showed that reducing the number of high humidity hours to 6 still gives ample blight protection even of highly susceptible potato cultivars. In 1995, Cao *et al*. (1997) looked at potato late blight epidemics on naturally infected field plots of the susceptible potato cultivar Charlotte and described 'crucial weather conditions' (CWC) for infection periods based on observations. Daily counts of new lesions were used to assess the disease severity and artificial inoculations were used to assess LP. Weather data on these days were used to create the CWC which were stated to be 6 hours of rain with an air temperature of 10°C or above and a minimum of 6 uninterrupted hours of 90% RH or above over 24 hours. In the following year, field experiments on Bintje were used to validate the CWC model and to compare it to NegFry (discussed in Chapter 1.13) and PhytoPre system. When using the CWC model, three fungicide applications were used and this was enough to give adequate protection to the crop; The PhytoPre system recommended six fungicide applications but gave the same level of protection. NegFry recommended only two fungicide applications but this did not give adequate protection to crop. Although the CWC-model required more fungicide application than NegFry, it gave better protection and when comparing the CWC-model to PhytoPre system less fungicide was used to get the same protection. Assessing epidemics for two seasons is not substantial enough to base a forecasting system on, but basing forecasting systems on biological parameters and meteorological parameters would hopefully give more accurate blight risk assessment.

6.4.4 Conclusion

To create an effective forecasting system both humidity and temperature need to be examined and the relationship between these two factors will affect the rules. This study shows that the humidity period affects the rate at which *P. infestans* infects and

causes disease. The humidity period needed for infection and colonisation is shorter than is stated in the Smith Period.

Chapter 7 - General discussion

7.1 Introduction

The main objective of this study was to assess the effect of *P. infestans* population change on late blight management in the UK, specifically whether the Smith Period is still a valid method of forecasting. This was done by assessing the biological parameters of 11 contemporary genotypes of *P. infestans* found in UK. In Chapter 3 the differences in aggressiveness between the genotypes on detached leaflets were described; the competitive ability of four genotypes on whole detached leaves and in a field epidemic was examined in Chapter 4; and the effect of the Smith Period criteria, temperature and humidity, on pathogen infection and growth was described in Chapters 5 and 6.

7.2 UK populations

Throughout the investigations reported here there has been a consistent trend that the isolates of genotype 13_A2 were not the most aggressive of the 11 UK genotypes compared in this study. Cooke *et al*. (2012a) indicated that six isolates of genotype 13_A2 tested in 2007 were amongst the three most aggressive genotypes and were more tolerant of the cooler (13°C) than the warmer (18°C) temperatures tested. On this basis it was hypothesised that this adaptation had allowed the genotype to become dominant within the population. This study, however, did not show such clear cut evidence. Isolates of genotype 13_A2 did not, on average, show any significantly greater aggressiveness than other genotypes at any temperature tested (Chapters 3 and 5). They did prove to have amongst the largest lesions at lower temperatures in the *in vivo* study (Chapter 5) but this difference was not statistically significant. Studies reported in Chapter 4 showed that genotype 6_A1 was more aggressive than genotype 13_A2 when looking at a single plant within the field plot.

Most tests in this study concentrated on aggressiveness over a single *P. infestans* life cycle from inoculation to sporulation. However, potato late blight is a polycyclic disease in which multiple disease cycles are completed in a single season. Fitness is the measure of the ability of an organism to survive, reproduce in the environment and contribute genes to the next generation (Orr, 2009). It is not determined by one factor but encompasses all aspects of the life cycle and the fitness of one genotype is not constant as the selection pressures of the environment change. Although aggressiveness and fitness are related, they are different as aggressiveness measures the amount of disease an organism can cause while fitness relates to transmission of inoculum from one generation to the next. Over multiple infection cycles (Chapter 4) genotype 13_A2 proved to be the fittest genotype out of those tested as it completely dominated the field plots even though in the laboratory it was not the most aggressive. The small differences seen in the laboratory which are classed as not significant could be having a great effect in a naturally occurring infection. It is easy to see how small differences between the single life cycles of genotypes could produce large differences in the field as the small differences will be applied to every infection, for example, if one lesion produced 10 sporangia that went on to create a lesion, and each of these lesions produced 10 more sporangia which created a lesion, after only two life cycles there would be 110 lesions. In practice this is magnified greatly as *P. infestans* produces in the region of 300 sporangia per mm^2 of a sporulating lesion (Skelsey *et al*., 2009). In this way slight non-significant differences seen within one life cycle are quickly multiplied. The diurnal experiment suggested that genotype 13_A2 was able to infect faster between the mean temperatures 10°C and 13°C, but these differences were very subtle and were not statistically significant (Chapter 5). If genotype 13_A2 causes sporulating lesions sooner by infecting faster between these temperatures, a small head start would be multiplied with every infection and could lead to an established

epidemic. The head start could be further enhanced as the LP and the lesion size of the isolate of genotype 13_A2 tested in the laboratory competition study (Chapter 4) was shown to be less affected that the other genotypes to the presence of a competitor.

The findings in the *in vivo* with diurnal patterns and the field trial were similar in regards to genotype 13_A2 being adapted to lower temperatures, as in Chapter 5 it showed a slight adaptation to the lower temperature combinations with a single life cycle and in Chapter 4 showed the consequence of that over multiple life cycles in a plot. The weather data in Belfast dropped below 10°C at several points at which genotype 13_A2 would have a slight advantage over other genotypes.

An important factor to remember is that genotype 13_A2 is metalaxyl-resistant. This was a key active ingredient in late blight management before the change in the population. When genotype 13_A2 was first detected metalaxyl was commonly used which would have given genotype 13_A2 a distinct advantage over some other genotypes. Metalaxyl was used intensively in the years 2004 and 2005 but usage has fallen gradually over the years until in 2010/2011 hardly any was used (Figure 7.1). This reduction in the use of metalaxyl was in response to reports of genotype 13_A2's insensitivity to metalaxyl (Shaw *et al*., 2007; Duvauchelle *et al*., 2008). The decline in genotype 13_A2 over this period may thus be partly explained by the removal of the selection pressure of metalaxyl application. There are several reports of the proportion of metalaxyl resistant strains reducing in line with a reduction in metalaxyl usage (Holmes and Channon, 1984; Dowley and O'Sullivan, 1985; Cooke and Little, 2006). However, these reports relate to metalaxyl resistant genotypes present in the population before 2005. Metalaxyl resistant lineages that have arisen since 2005 (Shaw *et al*., 2007) did not go on to become dominant within the UK population. This further supports the suggestion that metalaxyl insensitivity was a factor, but not the only factor, that explains the dominance of 13_A2.

There were genotypes that were more aggressive than genotype 13_A2 in the tests reported here, but this aggressiveness was not expressed in the UK populations. Genotype 2_A1 proved to be highly aggressive in several of the tests described here; much more so than genotype 13_A2 in some cases. In 2003 and 2004, genotype 2_A1 was prevalent in the UK population but rapidly decreased in numbers when genotype 13_A2 emerged. It may not have been able to maintain its position within the UK population due to genotype 13_A2 being fitter (Chapter 4 and 5) and the increased use of metalaxyl in 2004 and 2006, thus being out competed and reducing in frequency. Genotype 6_A1 was the most aggressive genotype tested in the field trial and in the natural environment it has managed to maintain a prominent place in the UK populations, even dominating the population in 2011 (D. Cooke personal communication). Its ability to compete with genotype 13_A2 could be due to it being highly aggressive but this cannot be the only factor as genotype 2_A1 is just as aggressive (in the laboratory experiments). Genotype 6_A1 did produce significantly larger lesions on Lady Balfour in the aggressiveness tests (Chapter 3, Test 2) so the fact it can overcome previously resistant cultivars, much like genotype 13_A2, may be a factor in maintaining a position in the UK populations (Lees *et al*., 2012). Unlike genotype 13_A2, genotype 6_A1 is metalaxyl-sensitive so perhaps the increase in genotype 6_A1 in the UK population relates more to the reduction in the use of metalaxyl rather than its ability to overcome cultivar resistance. In 2011, the UK *P. infestans* populations underwent another dramatic change this time involving genotype 6_A1. Genotype 6_A1 rose steadily in the population from 2004 but never dominated the GB population until 2011 (Cooke, D.E.L., pers. comm.) when there was a concomitant decline in 13_A2. This could be due to two factors. Sample bias could play a role as in 2011 a lot of the samples taken for the Fight Against Blight survey (discussed in Chapter 1.15) were from Scotland, while very few samples came from

England (Cooke, D.E.L., pers. comm.). However, in Northern Ireland genotype 13_A2 also declined in the population in 2011 and was displaced not by genotype 6_A1 but by genotype 8_A1 (Cooke *et al.*, 2012b). That a decline in genotype 13_A2 occurred across Ireland as well as GB suggests that the result was not due to sample bias. This leads on to the other factor that could explain the dominance; the weather. In Scotland, it was a very warm and wet spring in 2011. Data in this study showed that at 15°C genotype 6_A1 had a higher AULEC value (thus larger lesions) than genotype 13_A2. The meteorological data showed that in April and May 2011 in the UK the temperature was above the average, as much as 4°C in April and 1.3°C in May (Anon., 2012, Appendix i). In June, the temperature was just above average but even so this increase would mean that genotype 6_A1 would have an advantage over genotype 13_A2 especially because the previous months were warmer, thus giving 6_A1 a better chance to initiate the primary infection.

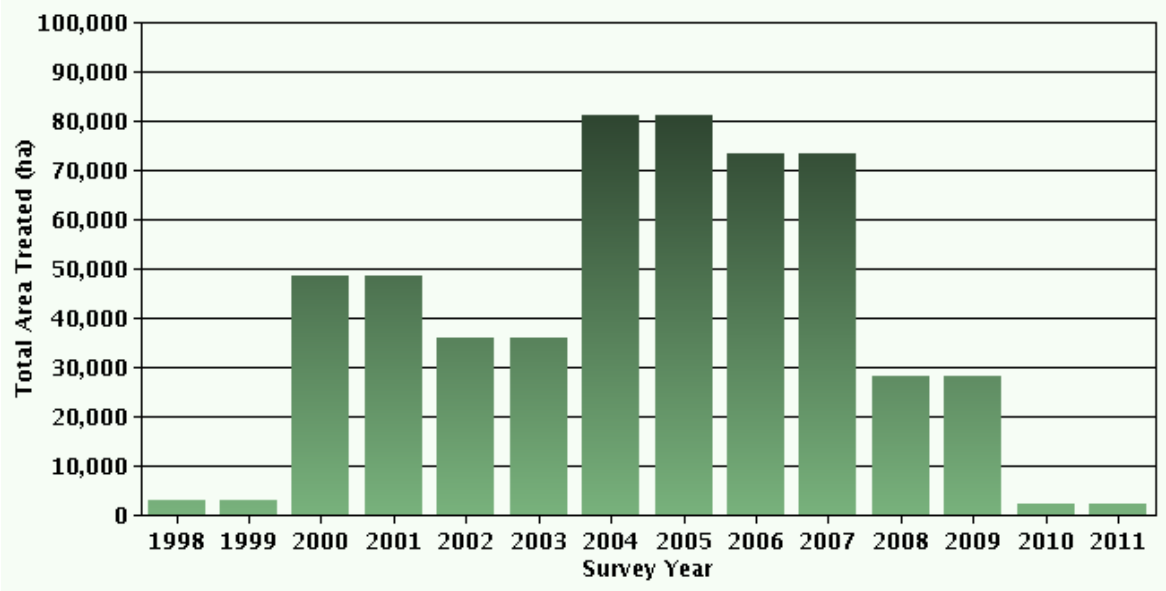


Figure 7.1 – The total area of potato fields treated with metalaxyl-M between the years 1998 and 2011. Usage in 1998 and 1999 is low as during these years metalaxyl was widely used and this data is not accounted for in this figure, metalaxyl-M replaced metalaxyl in the 2000s. Data taken from the Fera pesticide usage website

Variation within genotypes was an unexpected finding in this thesis, although some variation within a genotype is expected, the extent to which this was seen in this study was surprising. It is difficult to distinguish between variation due to test differences and genuine differences between the isolates/genotypes. Many factors affect the variation that is seen between tests such as inoculum, leaf age and environmental conditions. Lehtinen *et al*. (2009) found that even when aggressiveness tests were standardised the amount of variation seen was large and thought to be due to the laboratory effects (discussed in Chapter 3.4.1). Comparing between tests introduces most problems as generally, each test would be conducted at different times with different plants and different inoculum. With the aggressiveness test in Chapter 3, the tests were done at different times of the year using inoculum that had been passaged through more life cycles each time, thus more mitotic events occurred and consequently more variation might have been introduced. Concentrating on the standard isolates used in the aggressiveness test, the difference in the lesion size between the four separate experiments is large, especially for isolate 2007_5442F in which the lesion size ranged from 8.96 mm^2 to 16.37 mm^2. The range in lesion size for isolate 2006_3928A was smaller with only Test 4 being significantly different from the other tests; the range was 8.23 mm^2 to 11.82 mm^2. Standard isolates have been shown to give significantly different values in different tests (Day and Shattock, 1997), but interestingly in the *in vivo* experiment the standard isolate values did not show significant variation between most temperatures. Only isolate 2006_3928A (genotype 13_A2) was used and this isolate showed the least amount of significant variation between the aggressiveness tests. So when comparing aggressiveness tests, the isolate(s) that are chosen to be the standard isolate(s) (if using this method) will affect the variation seen between tests. Variation within the experiments is more likely to relate to real differences between isolates as there would be less variation in leaf age and inoculum preparation. Using

the standard isolates of the aggressive study in Chapter 3 as an example, the standard errors seen for each test were relatively small (SE=0.49) showing that the amount of variation between the replicates within the test was small. Multiple replications within the tests are important as when statistically analysed the amount of inherent variation seen within the isolates can be estimated and taken into account. Cooke *et al*. (2010) stated that the large number of replicates in the aggressiveness test conducted in 2007 were valuable because the ANOVA indicated that there were highly significant differences between isolates.

The lack of reproducibility makes it difficult to be able to associate a particular level of aggressiveness with a genotype. Genotype 17_A2 is a prime example of this; in the *in vivo* study it proved aggressive and was selected for the diurnal experiment in which it was the least aggressive. In the aggressiveness test, some genotypes did not have consistent rankings between the tests and this may be due to the isolates being tested, for example, one test could have contained the more aggressive isolates of a genotype whereas the other tests could have contained the least aggressive isolates. It is difficult to generalise and state a level of aggressiveness for any single genotype from such laboratory tests. This is particularly true when focusing on one life cycle as shown by genotype 13_A2 as the true extent of its fitness was masked. Using a combination of laboratory tests and field trials could be the way forward in assessing the aggressiveness of a genotype as in the field trial, three variants of genotype 13_A2 were used to make the mixed inoculum, and one of the variants, genotype 13_A2_5 was not recovered from the plot. This is the variant that was the least aggressive in the studies of Chapter 5.

A1 Misc and A2 Misc was each a composite of novel genotypes found within the UK populations that were genetically distinct from the common *P. infestans* genotypes found previously. Both A2 Misc and A2 Misc had colony sizes and lesion sizes that

ranged from large to small, the only real differences were seen at the lower temperatures where an isolate of the A2 Misc had a large lesion size at 6°C in the *in vivo* study but in the *in vitro* study, hardly any of the isolates of genotype A2 Misc grew. The variation in aggressiveness of these two genetically diverse groups was similar to the variation seen within the other genotypes. It may be hypothesised that isolates of the 'misc' remain at a low frequency in the population due to lower aggressiveness than the dominant clonal lineages but the evidence presented here contradicts this as it suggests that they have aggressiveness within the normal range of the population.

7.3 The Smith Period

A Smith Period states that there is a risk of blight if inoculum is present when on two consecutive days the minimum temperature was 10°C and there were at least 11 hours of 90% RH on each day (Smith, 1956). The Smith Period was based on weather data and epidemiological patterns seen in the 1950s. Although it does reliably predict blight in an area, care must be taken when using the Smith Period as a forecasting system as the minimum temperature cut off point is illogical when looking at biological data. Growth will still occur under 10°C (Crosier 1934 ; Rotem 1970) with the majority of the 11 UK genotypes tested here being able to infect at 8°C (Chapter 5.2.3). Basing a forecasting system on weather alone does bring its own set of problems. Smith periods are calculated on the basis of data interpolated from a network of meteorological stations, but the accuracy of any Smith Period prediction would depend on how far away the weather station collecting the data for the region is from a field (Taylor *et al*., 2002). Even sensors within a field could provide incorrect readings due to sheltering effects of trees (Taylor *et al*., 2002). Barrie and Bradshaw (2002) stated that because of introduction of the new genotypes of both mating types (Spielman *et al.*, 1991) the Smith Period maybe not be suitable mainly due to the complicated interactions of the

pathogen and environment and the unknown growth parameters of the newer genotypes. This was stated before genotype 13_A2 was detected within the population. Before genotype 13_A2, missing a few critical periods may not have carried higher risks (Flier *et al*., 2002), but now, with a contemporary population that contains fitter and aggressive genotypes failing to predict the first critical periods may lead to severe blight pressure for the grower (Flier *et al*., 2002).

Running experiments at fixed temperatures is never going to simulate the natural environment of a potato crop. In the aggressiveness test and the *in vivo* test all temperatures were constant with 100% RH, and even the diurnal experiment which looks at how differing day and night temperatures affect growth is not truly representative of what would occur in a crop. The effects of the complex interactions of the environmental conditions upon the growth of the crop and *P. infestans* are likely to be subtle. All tests are important though, even if they do not simulate the exact conditions within a crop because they provide growth parameters which are highly important when it comes to creating an accurate and effective forecasting system. *Phytophthora infestans* is not going to encounter 7 days at 6°C or 15°C but studying the growth at different constant temperatures allows a broader understanding of growth responses. Also, dominance may not be attributed to just one factor, such as being highly aggressive at one temperature, so looking at different temperatures and how they relate to each other gives the bigger picture. There needs to be a method of using the data collected to put in perspective how the growth at certain temperatures would affect the disease development within an epidemic and this is done by using disease epidemic modelling.

The multi-scale potato late blight model of Skelsey *et al.* (2010) is an adaptation of the validated Skelsey *et al*. (2009) model discussed in Chapter 1.11. The model simulates disease epidemics on a 2D grid in which one cell of the grid represents a potato field.

All of the cells that make up a grid create a landscape that are linked together with spore dispersal and survival models and the landscape goes through multiple growing seasons. The model manipulates the composition, configuration and the connectivity of the potato fields within the landscape to study how this influences disease spread.

A scenario study was conducted using the Skelsey *et al*. (2010) model that simulated a competitive situation between genotype 13_A2 and 6_A1 within a landscape, using weather data used were collected from Balruddery Farm, Perthshire, UK in 2010. Empirical values for lesion growth rate were genotype specific and temperature dependent; taken from the *in vivo* study in Chapter 5. LP was set to 4 days for genotype 6_A1 and 5 days for genotype 13_A2 and other parameters were set as in Skelsey *et al*., (2009). Smith Periods within the model were based on the weather data which governs disease development as it acts as an on/off switch; if there is a Smith Period, sporangia will disperse and germinate to cause more lesions but if there is no Smith Period only growth of the lesion occurs. Two scenarios differing only in the temperature rule of the Smith Period were run; firstly, the rules were set to the existing Smith Period i.e. the minimum temperature must be 10°C or above, and the second run stated that the minimum temperature must be 6°C or above (as described in Chapter 5.3.2). When the standard Smith Period was used in the model, genotype 6_A1 had a higher incidence level at all levels of field distribution when compared to genotype 13_A2 (Figure 7.2). Conversely, when the modified Smith period was implemented by the model, it was genotype 13_A2 that had the higher incidence particularly as the level of distribution generated clusters of fields rather than randomly distributed fields (P. Skelsey, personal communication). Further work on the humidity criteria is required to see how the proposed 6 hours RH stated in Chapter 6 would affect the simulated scenario.

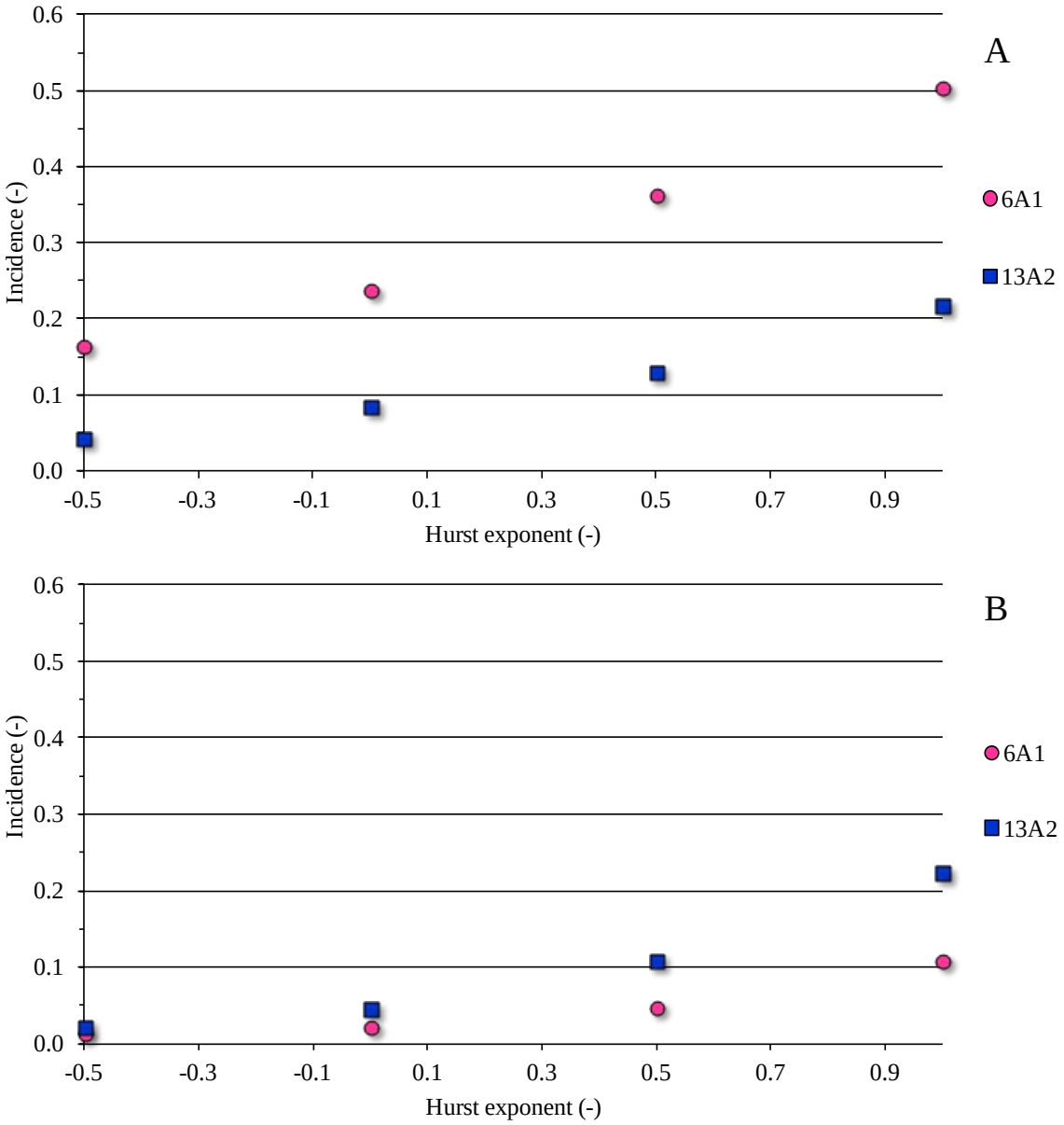


Figure 7.2 – A simulated level of incidence seen for genotypes 13_A2 and 6_A1 for different levels of potato field distribution within a landscape when ran in Skelsey's *et al* (2010) multi-scale potato late blight model at different Smith Period criteria.
A) Incidence seen when sporulation is governed by the Smith Period i.e. sporulation only occurs when the minimum temperature is 10°C for two days and the RH is 90% for hours on each day.
B) Incidence seen when sporulation is governed by the Modified Smith Period i.e. sporulation only occurs when the minimum temperature is 6°C for two days and the RH is 90% for 11 on each day.

7.4 Conclusion

The scenario shows that because of genotype 13_A2 there needs to be a change in how the UK potato industry manages potato late blight in regards to a forecasting system. The Smith period was never meant to be used as a precise spray decision tool but to give an indication of when there is a potential risk of blight in a particular area (Barrie and Bradshaw, 2002). This study has shed light on the need for a change in order to better protect potatoes from a more aggressive and fitter pathogen population than that present in the 1950s when the Smith Period was defined. It is increasingly important to monitor the UK *P. infestans* populations as change can happen very suddenly with drastic repercussions; as seen when genotype 13_A2 first emerged. Further research could provide information about the subtlety of growth with changing temperatures and humidity and provide more knowledge for increasing the accuracy of forecasting systems. Information about the biological parameters needs to be fed into a more robust DSS model that integrates aspects such as pathogen growth, host growth, host resistance and fungicide usage and weather (past and forecast) to provide better crop protection advice to growers.

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Appendix i

Appendix i - Average weather data for the UK between March and June for years 1981-2010 (meaned over the years) and 2005-2011 (mean for each separate year). Data collected by the Meteorological Office.

Month	Year	Max Temp. (°C)	Min Temp. (°C)	Mean Temp. (°C)	Rainfall (mm)
March	1981-2010	8.9	2.1	5.5	95.1
March	2005	9.6	3.2	6.4	73.3
March	2006	7.1	0.8	3.9	110.9
March	2007	10.1	205	6.3	88.9
March	2008	8.5	1.8	5.1	122.5
March	2009	9.9	2.3	6.1	79.5
March	2010	8.9	1.3	5.1	79.4
March	2011	9.8	1.9	5.8	49.7
April	1981-2010	11.4	3.4	7.4	148.1
April	2005	11.9	3.9	7.9	91.2
April	2006	11.3	3.5	7.4	67.8
April	2007	15.2	5.3	10.2	27.0
April	2008	11.1	3.1	7.1	76.2
April	2009	13.3	4.6	8.9	60.2
April	2010	12.7	3.3	8.0	48.0
April	2011	15.7	5.7	10.7	36.7
May	1981-2010	14.7	6.0	10.3	185.9
May	2005	14.4	5.7	10.0	72.9
May	2006	15.0	6.5	10.7	112.5
May	2007	14.8	6.5	10.7	114.1
May	2008	16.8	7.6	12.2	47.9
May	2009	15.3	6.5	10.9	82.0

Appendix i continued

Month	Year	Max Temp. (°C)	Min Temp. (°C)	Mean Temp. (°C)	Rainfall (mm)
May	2010	14.4	5.2	9.8	39.0
May	2011	15.2	6.9	11.0	101.8
June	1981-2010	17.3	8.8	13.0	169.5
June	2005	18.5	9.8	14.1	72.0
June	2006	19.4	9.7	14.5	42.4
June	2007	17.7	9.9	13.7	136.2
June	2008	17.3	8.7	12.9	78.5
June	2009	18.4	9.0	13.7	62.1
June	2010	19.1	9.3	14.2	38.6
June	2011	17.1	8.3	12.7	85.1

Appendix ii

Appendix ii – List of lesion sizes, IP and LP for *P. infestans* isolates in all tests of the aggressiveness study

LS Exp 1	Cultivar P=0.92 SE=3.52				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	19.85	19.81	22.79	22.06	24.25
2006_3984C	21.87	20.14	26.17	25.65	27.39
2007_5074E	20.02	24.90	25.44	20.17	24.52
2007_5442F	25.37	20.80	33.28	22.21	27.53
2007_5726C	8.29	12.95	19.54	10.94	20.39
2008_6050B	21.45	16.73	24.40	27.47	22.44
2008_6070E	19.02	14.26	25.83	19.32	19.96
2008_6090A	24.69	22.96	28.22	29.35	28.00
2008_6102A	18.25	22.04	22.66	21.84	23.07
2008_6194A	17.71	19.28	21.01	20.03	19.27
2008_6354C	24.14	20.38	23.31	22.03	25.29
2008_6394B	12.75	11.86	17.97	13.32	16.46
2008_6426A	21.27	22.76	25.38	23.86	28.69
2008_6502A	24.30	23.05	26.23	22.84	24.64
2008_7006D	17.18	13.94	19.84	17.32	21.16
2008_7038A	19.00	19.77	21.89	21.92	23.41
Bayer 9B	20.88	21.26	29.10	24.06	28.41

LS Exp 2	Cultivar P<.001 SE=2.83				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	18.15	19.19	19.00	20.74	24.66
2006_4232E	3.59	14.44	19.64	11.94	18.94
2007_5054A	23.22	21.83	26.14	23.08	26.89
2007_5442F	27.35	30.13	29.42	25.92	33.71
2008_6306A	9.60	11.79	14.80	17.45	17.64
2008_6446F	2.69	20.24	22.50	20.07	29.07
88069	4.66	4.07	11.96	11.61	21.65

Appendix ii continued

LS Exp 3	Cultivar P=0.01 SE=3.06				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
07/39	6.04	13.72	14.80	12.12	9.87
2006_3928A	13.93	18.98	18.83	15.65	20.13
2006_4012F	10.97	11.56	14.10	15.37	13.37
2006_4100A	1.97	1.13	0.91	2.16	1.87
2006_4168B	1.65	8.16	11.95	10.40	11.93
2007_5138G	17.96	18.04	17.86	17.02	15.11
2007_5290C	2.13	16.75	23.15	11.54	14.99
2007_5442F	16.92	20.33	18.96	19.93	17.28
2007_5482D	16.00	23.19	20.25	22.58	22.61
2007_5482E	16.24	24.95	19.87	23.14	16.93
2007_5622A	13.34	17.54	15.69	18.37	14.58
2007_5738G	14.46	17.78	21.08	15.37	15.82
2008_6090A	16.19	25.71	21.71	20.16	23.73
2008_6222A	1.96	4.85	6.74	3.90	10.10
2008_6274D	19.19	23.80	22.60	23.64	19.31
2008_6422F	10.01	9.12	17.56	13.05	10.03
2008_6430A	10.04	10.07	10.90	12.92	13.50
2008_6530C	5.03	2.71	4.05	5.15	2.75
2008_6610E	7.61	9.08	11.442	11.34	13.39
2008_6850D	15.53	21.91	16.88	16.64	17.48

LS Exp 4	Cultivar P<.001 SE=2.05				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	20.19	21.21	24.84	23.56	23.21
2006_4388C	1.57	8.75	11.04	2.54	2.14
2008_4388D	19.29	24.50	23.59	25.28	24.75
2006_4440C	22.17	20.29	27.42	23.45	22.58
2007_5442F	21.85	23.83	24.32	23.54	25.24
2007_5918A	11.79	11.62	21.76	14.88	19.25

Appendix ii continued

2007_5974A	18.88	19.97	21.49	22.68	21.88
2008_6082F	18.82	21.19	24.59	24.89	23.39
2008_6066A	1.04	5.75	6.21	4.90	0.74
2008_6090A	20.51	22.11	27.25	26.92	26.83
2008_6250D	13.01	14.78	20.62	18.45	18.53
2008_6458E	16.04	17.64	21.43	19.95	19.26
2008_6498A	0.71	7.85	4.53	13.73	10.94

IP Exp 1	Cultivar P=1.00 SE=0.34				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	3.3	3.8	3.6	3.3	3.5
2006_3984C	3.0	3.3	3.1	3.0	3.1
2007_5074E	3.5	3.3	3.3	2.6	3.0
2007_5442F	2.8	3.1	3.1	3.1	3.0
2007_5726C	3.7	3.5	3.6	3.6	3.6
2008_6050B	3.3	3.8	3.6	3.3	3.6
2008_6070E	3.3	3.6	3.3	3.1	3.5
2008_6090A	3.1	3.1	3.1	3.1	3.0
2008_6102A	3.0	3.3	3.0	3.1	3.0
2008_6194A	3.8	4.0	3.6	3.6	4.0
2008_6354C	3.1	3.3	3.5	3.0	3.0
2008_6394B	3.8	4.3	4.3	4.2	4.0
2008_6426A	3.0	3.0	3.0	2.8	3.0
2008_6502A	3.2	3.6	3.0	3.1	3.1
2008_7006D	3.3	3.8	3.8	3.8	3.6
2008_7038A	3.1	3.6	3.5	3.3	3.5
Bayer 9B	3.3	3.6	3.8	3.1	3.5

Appendix ii continued

IP Exp 2	Cultivar P=0.01 SE=0.27				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	3.6	3.5	3.6	3.6	3.2
2006_4232E	2.6	3.1	3.0	3.0	3.0
2007_5054A	2.8	3.0	3.8	3.1	3.0
2007_5442F	2.1	2.5	2.6	2.8	2.8
2008_6306A	4.2	3.9	4.5	3.5	3.5
2008_6446F	2.6	3.0	3.1	3.0	3.0
88069	3.2	3.2	3.1	3.0	3.0

IP Exp 3	Cultivar P<.001 SE=0.27				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
07/39	3.5	3.5	3.5	3.6	3.8
2006_3928A	3.0	3.3	3.0	3.0	3.3
2006_4012F	4.5	3.6	4.3	4.0	3.3
2006_4100A	4.0	5.9	4.5	4.2	4.9
2006_4168B	3.4	3.3	3.2	2.8	3.0
2007_5138G	2.8	3.1	3.0	3.1	3.1
2007_5290C	3.8	3.1	3.3	2.8	3.1
2007_5442F	3.2	3.2	3.0	3.0	3.0
2007_5482D	3.5	3.6	3.5	3.3	3.1
2007_5482E	3.0	3.3	3.3	3.0	2.8
2007_5622A	3.0	3.0	3.8	3.4	3.6
2007_5738G	3.1	3.0	3.5	2.8	3.3
2008_6090A	3.0	3.3	3.0	3.0	3.0
2008_6222A	3.2	3.6	3.0	2.8	3.1
2008_6274D	3.2	3.6	3.0	2.8	3.1
2008_6422F	3.2	4.0	4.0	3.3	3.4
2008_6430A	3.5	3.7	3.6	3.6	3.1
2008_6530C	4.6	3.6	4.7	4.4	4.5
2008_6610E	3.4	3.3	3.5	3.3	3.0
2008_6850D	3.1	3.0	3.0	2.8	2.8

Appendix ii continued

IP Exp 4	Cultivar P<.001 SE=0.22				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	3.0	3.0	3.0	3.0	3.0
2006_4388C	3.9	3.9	4.0	3.6	4.0
2008_4388D	3.1	3.0	3.5	3.0	3.0
2006_4440C	3.0	3.0	3.0	3.0	3.3
2007_5442F	3.1	3.0	3.0	3.0	3.0
2007_5918A	3.1	3.0	3.3	3.0	3.0
2007_5974A	3.0	3.1	3.3	3.0	3.0
2008_6082F	3.0	3.0	3.1	3.0	3.0
2008_6066A	3.9	6.8	4.0	4.6	4.0
2008_6090A	3.0	3.0	3.0	3.0	3.0
2008_6250D	3.0	3.0	3.1	3.0	3.0
2008_6458E	3.1	3.5	3.3	3.0	3.0
2008_6498A	4.4	3.1	3.6	3.2	3.0

LP Exp 1	Cultivar P=0.39 SE=0.64				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	6.3	5.2	5.8	7.0	6.6
2006_3984C	6.0	5.5	5.3	5.6	5.3
2007_5074E	5.8	5.5	5.8	7.1	5.6
2007_5442F	6.3	6.0	6.0	7.5	6.0
2007_5726C	7.9	6.8	6.3	8.0	6.3
2008_6050B	5.8	5.4	5.5	5.5	5.5
2008_6070E	5.6	6.5	5.8	7.5	5.6
2008_6090A	5.5	5.5	5.3	6.6	5.0
2008_6102A	6.5	5.0	5.0	5.8	5.1
2008_6194A	6.0	5.4	6.0	7.6	5.0
2008_6354C	5.0	5.0	5.5	6.8	4.0

Appendix ii continued

2008_6394B	6.1	5.8	5.3	7.2	5.8
2008_6426A	4.8	5.6	5.5	5.8	5.3
2008_6502A	6.2	5.6	5.5	5.5	5.1
2008_7006D	6.3	6.8	6.4	7.6	6.5
2008_7038A	5.5	4.8	5.6	6.1	5.6
Bayer 9B	6.0	6.3	5.5	7.0	5.8

LP Exp 2	Cultivar P<.001 SE=2.83				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	5.8	5.3	5.4	5.6	5.5
2006_4232E	8.0	6.1	5.1	7.3	5.6
2007_5054A	5.0	5.1	5.0	5.1	4.6
2007_5442F	5.6	5.5	5.1	5.3	4.8
2008_6306A	7.0	6.4	6.5	6.0	6.2
2008_6446F	8.0	5.8	5.3	5.6	4.8
88069	8.0	8.0	7.5	7.0	6.0

LP Exp 3	Cultivar P=0.04 SE=0.53				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
07/39	7.2	5.8	6.0	6.6	6.8
2006_3928A	5.8	5.5	5.3	5.3	6.0
2006_4012F	6.5	7.4	6.6	6.3	6.3
2006_4100A	8.0	7.9	7.5	7.5	7.9
2006_4168B	8.0	7.5	6.4	6.6	6.8
2007_5138G	6.6	5.0	5.1	5.3	5.5
2007_5290C	8.0	7.0	6.0	7.5	5.6
2007_5442F	6.4	6.0	5.0	5.5	5.4
2007_5482D	6.6	5.0	5.0	4.8	5.3
2007_5482E	6.6	5.5	5.6	5.1	5.3
2007_5622A	7.0	6.2	6.0	6.0	6.2
2007_5738G	7.3	6.0	5.6	6.0	5.8

Appendix ii continued - List of lesion sizes, IP and LP for *P. infestans* isolates in all tests of the aggressiveness study

2008_6090A	5.6	4.8	4.8	5.1	5.3
2008_6222A	7.5	8.0	7.1	7.4	6.8
2008_6274D	6.5	4.5	5.1	5.4	5.3
2008_6422F	7.5	8.0	6.2	7.0	7.6
2008_6430A	7.3	6.7	7.6	6.6	6.5
2008_6530C	8.0	8.0	7.5	7.0	7.5
2008_6610E	7.8	6.6	6.6	6.6	7.5
2008_6850D	6.3	5.1	5.3	6.1	5.6

LP Exp 4	Cultivar P=0.56 SE=0.56				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	5.3	6.1	4.1	5.1	4.8
2006_4388C	7.9	7.9	6.5	6.6	8.0
2008_4388D	5.1	5.6	5.0	5.1	5.0
2006_4440C	5.0	5.1	4.5	4.3	4.8
2007_5442F	6.1	5.8	5.8	6.1	5.5
2007_5918A	7.8	7.6	5.5	7.1	6.8
2007_5974A	6.1	6.0	4.8	5.3	5.6
2008_6082F	5.0	5.3	4.6	5.5	5.0
2008_6066A	7.9	7.9	8.0	7.8	8.0
2008_6090A	6.1	6.1	5.0	5.1	5.0
2008_6250D	7.8	7.5	5.6	7.1	7.1
2008_6458E	5.6	5.5	5.0	5.0	5.6
2008_6498A	8.0	7.8	7.0	7.6	7.4

AULEC Exp 1	Cultivar P=0.95 SE=6.31				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	32.48	27.81	35.48	40.05	38.47
2006_3984C	38.17	34.73	46.05	43.85	46.97
2007_5074E	32.22	40.38	38.17	37.50	43.67
2007_5442F	46.47	36.78	57.32	45.85	55.09

Appendix ii continued

2007_5726C	11.99	23.99	30.36	20.62	31.04
2008_6050B	34.14	21.82	38.59	44.08	36.97
2008_6070E	33.49	26.12	37.73	35.90	32.75
2008_6090A	43.05	38.45	52.18	55.03	54.11
2008_6102A	34.79	36.94	42.42	42.84	39.69
2008_6194A	27.47	25.52	35.46	33.46	31.06
2008_6354C	40.55	35.13	40.89	40.41	43.77
2008_6394B	22.51	16.84	27.31	19.43	24.39
2008_6426A	97.39	40.39	43.97	44.12	52.17
2008_6502A	37.23	38.30	44.71	44.57	45.70
2008_7006D	28.88	22.08	28.94	25.06	33.10
2008_7038A	33.85	25.15	37.07	40.41	36.19
Bayer 9B	31.60	35.48	44.72	40.51	43.88

AULEC Exp 2 Cultivar P=<.001 SE=6.31

Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	32.67	32.14	33.16	37.85	27.67
2006_4232E	9.63	27.78	39.35	28.33	40.20
2007_5054A	45.20	46.51	50.98	50.66	56.91
2007_5442F	66.79	62.97	67.47	59.92	72.15
2008_6306A	12.43	7.10	21.41	28.13	20.54
2008_6446F	9.27	35.77	44.21	41.43	59.09
88069	8.82	8.33	22.31	20.91	35.00

AULEC Exp 3 Cultivar P=0.05 SE=6.00

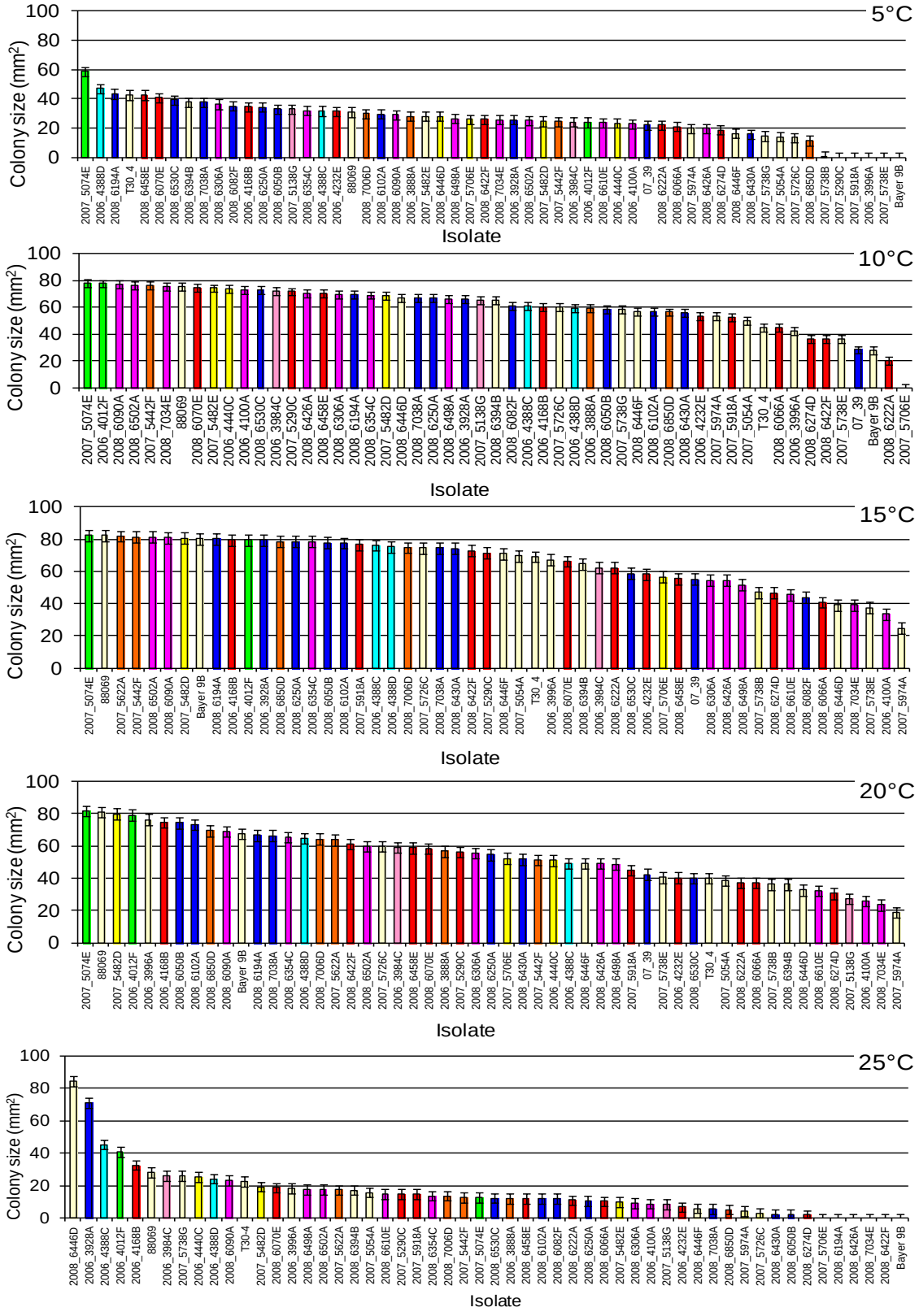
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
07/39	9.35	22.17	24.74	22.51	18.84
2006_3928A	25.70	35.23	32.71	34.69	31.05
2006_4012F	4.49	14.26	19.16	12.51	10.70
2006_4100A	2.09	0.33	0.36	0.00	0.00
2006_4168B	3.79	16.80	19.30	22.21	23.11

Appendix ii continued

2007_5138G	34.96	38.32	33.07	36.42	29.37
2007_5290C	5.04	36.29	40.37	25.32	27.96
2007_5442F	29.22	22.15	34.32	39.56	27.69
2007_5482D	23.32	43.14	35.06	48.53	36.70
2007_5482E	27.27	42.67	33.52	48.50	35.50
2007_5622A	7.28	20.67	22.01	29.09	21.67
2007_5738G	28.70	33.35	33.54	31.66	28.84
2008_6090A	36.00	45.52	37.15	43.35	40.35
2008_6222A	4.14	12.16	13.53	9.66	18.14
2008_6274D	38.42	42.66	41.32	42.92	38.11
2008_6422F	13.47	12.56	23.62	23.76	14.34
2008_6430A	20.54	15.01	19.98	24.11	26.09
2008_6530C	3.85	6.50	4.46	7.37	2.21
2008_6610E	12.51	17.73	20.84	21.20	24.33
2008_6850D	31.74	34.13	30.32	30.88	32.86

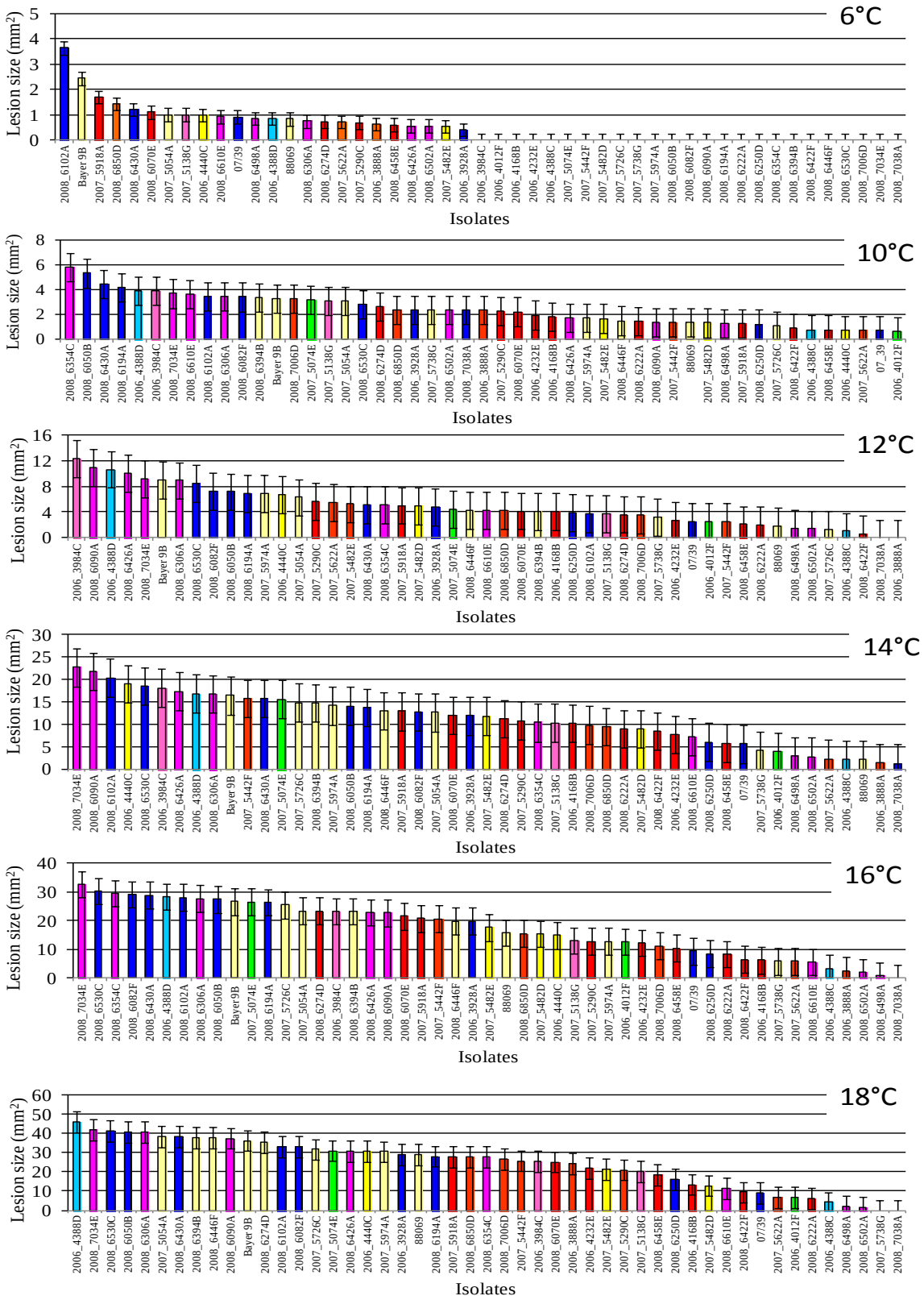
LP Exp 4	Cultivar P=0.07 SE=4.26				
Isolate	Cara	Estima	King Edward	Lady Balfour	Maris Piper
2006_3928A	41.14	41.15	52.53	49.87	48.71
2006_4388C	0.62	2.39	5.71	2.99	1.29
2008_4388D	38.79	51.62	48.11	51.44	50.21
2006_4440C	46.03	40.72	55.51	49.61	48.96
2007_5442F	47.04	49.66	50.65	51.16	55.25
2007_5918A	21.85	23.13	37.47	30.64	37.68
2007_5974A	35.34	36.40	44.38	45.32	45.89
2008_6082F	39.30	40.71	48.53	53.70	50.26
2008_6066A	0.47	0.86	3.39	0.00	0.77
2008_6090A	39.16	42.46	56.50	54.68	54.83
2008_6250D	23.66	25.52	33.78	32.18	33.89
2008_6458E	29.02	30.84	37.69	35.77	35.41
2008_6498A	1.88	12.10	5.32	18.95	15.65

Appendix iii



Appendix iii – *In vitro* growth UK *P. infestans* isolates on Rye A at temperatures ranging from 5°C-25°C

Appendix iv



Appendix iv - *In vivo* growth of UK *P. infestans* isolates on Maris Piper detached leaflets at temperatures ranging from 6°C-18°C

Appendix v

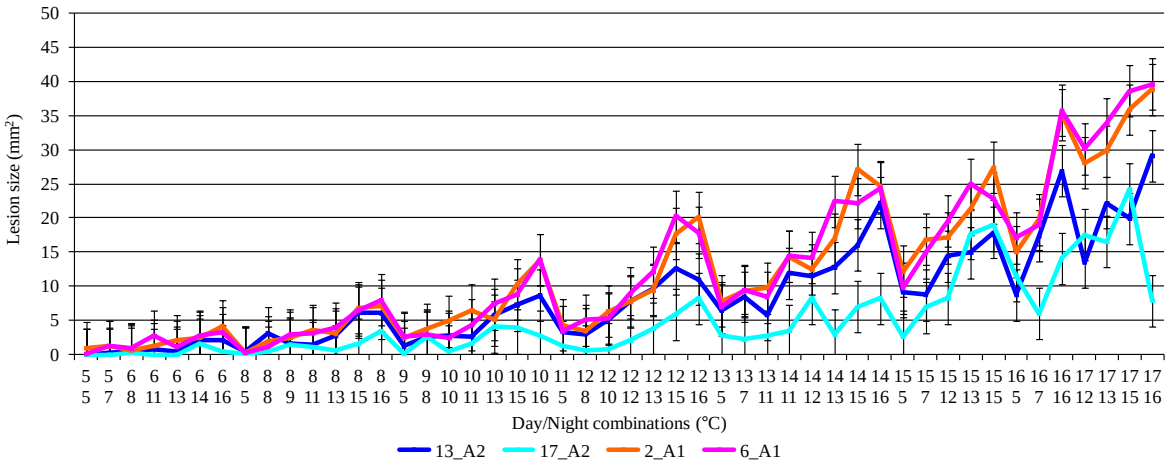
Appendix v – List of values from *in vivo* experiment of UK *P. infestans* on Maris Piper detached leaflets

Experiment	Isolate	IP-14°C	IP-16°C	LS-20°C
1	2006_3928A	4.2	4.0	26.27
1	2006_3984C	4.0	3.5	30.92
1	2007_5074E	4.5	4.0	32.18
1	2008_6070E	4.0	3.5	44.03
1	2008_6102A	4.0	3.5	35.41
1	2008_7006D	3.5	4.0	24.10
2	2007_5726C	6.0	6.0	36.83
2	2008_6354C	5.5	6.0	34.12
2	2008_6394B	6.0	5.0	42.85
2	2008_6502A	3.0	4.5	42.67
2	2008_7038A	4.0	4.0	1.29
2	Bayer 9B	4.5	4.0	37.53
3	2006_4232E	4.0	3.0	34.50
3	2007_5054A	4.0	3.0	43.14
3	2008_6446F	4.0	4.0	37.79
4	2007_5138G	5.5	3.0	35.11
4	2007_5290C	5.0	3.0	31.55
4	2007_5482E	3.0	2.5	41.55
4	2008_6274D	3.5	3.5	35.10
4	2008_6498A	5.5	7.0	8.77
4	2008_6610E	4.0	3.5	19.93
4	2008_6850D	3.5	2.5	42.59
5	07_39	3.5	3.0	11.27
5	2006_4012F	7.0	4.0	7.81
5	2006_4168B	5.5	3.0	6.26

Appendix v continued

Experiment	Isolate	IP-14°C	IP-16°C	LS-20°C
5	2007_5482D	3.5	3.0	12.15
5	2007_5622A	7.0	3.5	7.13
5	2007_5738G	7.0	7.0	5.91
5	2008_6422F	4.0	4.0	10.60
6	2006_3888A	4.0	7.0	6.12
6	2008_6050B	5.0	3.5	49.36
6	2008_6194A	3.5	3.0	45.81
6	2008_6306A	3.0	3.0	49.59
6	2008_6530C	5.5	4.0	42.67
6	2008_7034E	5.5	3.5	46.19
7	88069	5.5	3.5	38.08
7	2006_4388D	3.5	3.0	45.27
7	2008_6082F	5.5	3.5	49.97
7	2008_6222A	4.0	4.0	5.88
7	2008_6250D	5.5	5.5	28.37
7	2008_6430A	3.0	3.0	50.34
7	2008_6458E	5.5	5.5	25.76
8	2006_4388C	3.0	5.5	13.43
8	2006_4440C	5.0	3.0	39.63
8	2007_5442F	2.5	4.0	37.18
8	2007_5918A	3.0	2.5	37.16
8	2007_5974A	5.5	5.0	40.62
8	2008_6090A	3.5	3.5	39.49
8	2008_6426A	3.0	3.5	38.47

Appendix vi



Appendix vi – Mean lesion size (mm2) for four genotypes infecting Maris Piper detached leaflets at a range of different temperature combinations (SE=3.755)